PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter

November 7, 2012

Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

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welcomes you to our

PHI ZETA RESEARCH DAY FORUM
November 7, 2012

We want to thank all the presenters, their co-investigators and mentors for their participation in this annual event.

We also want to thank all sponsors for their generous support without which this event would not be possible:

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PHI ZETA RESEARCH EMPHASIS DAY

November 7, 2012 – Overton-Goodwin Center

Graduate Student Platform Presentations (OEW)

OEW 248		OEW 251	
8:30	Amy Back	8:45	Allison Bradbury
8:45	Michelle Aono	9:00	Erfan Chowdhury
9:00	Heather Davis	9:15	Elaine Norton
9:15	Elizabeth Barrett	9:30	Fernanda Cesar
9:30	Hui Huang	**9:45**	**Break**
9:45	**Break**	10:00	Anil Poudel
10:00	Valeria Albanese	10:15	India Napier
10:15	Kh. S. Rahman	10:30	Rucha Gurjar
10:30	Margaret Salter	10:45	Victoria McCurdy
10:45	Wes Campbell		

11:00-1:00 Poster Presentations- Overton Education Wing

(Poster Session Presenters are present 11:00 – 12:00)

Veterinary Student Platform Presentation (OEW 248)

1:30 Jeremy Fleming

1:45 Amelia Nuwer

2:00 Hannah Findlay

2:15 Kelsie Theis

2:30 Nikki McAdams

Post-graduate / Faculty Platform Presentations (OEW 248)

2:45 Deepa Bedi

3:00 Heather Gray-Edwards

3:15 Merrilee Holland

3:30 Jeremy Foote

3:45 Snack Break – Joy Goodwin Cafeteria

4:00 Keynote Lecture – Overton Auditorium – Dr. Greg Barsh

Genetics of color variation: Model organisms in a post-genome world

Greg Barsh, PhD

Greg Barsh is an Investigator at the HudsonAlpha Institute for Biotechnology in Huntsville, Alabama, and Professor of Genetics (emeritus) at Stanford University School of Medicine. Dr. Barsh received an MD and PhD from the University of Washington, obtained postgraduate training at UC Los Angeles and UC San Francisco, and has served as director of the Stanford Medical Scientist Training Program, and chairperson of the NIH study section on Genetics of Health and Disease. His research program is focused on the genetic architecture of color variation in laboratory mice and in natural populations of mammals, including humans. His research accomplishments based on the application of mouse coat color genetics to basic problems in cell signaling, gene regulation, and human disease have been recognized by several awards, including the E Mead Johnson Award from the Society of Pediatric Research, the Takeuchi and Seiji Awards from the International Society for Pigment Cell Research, and the Research Achievement Award from the American Skin Association. He is currently the president of the PanAmerican Society for Pigment Cell Research, and Editor in Chief of PLoS Genetics.

6:30 Phi Zeta Banquet at the AU Hotel & Dixon Conference Center

PLEASE JOIN US FOR THE INDUCTION AND AWARDS BANQUET

Everybody is invited! Reserve ticket by November 2, 2012 with Dr. Missy Josephson (josepem@auburn.edu, 334-844-5423). Deposit check for ticket with Dr. Josephson, 109 Greene Hall, or at the banquet in the **Auburn University Hotel & Dixon Conference Center**.

6:30 **COCKTAILS at cash bar**

7:00 **DINNER**

7:30 **INDUCTION of new Phi Zeta Members**

AWARDS CEREMONY to honor winners of the Platform and Poster Competitions

POSTERS

Veterinary Students

Caitlin Trebelhorn	Stearidonic acid, an omega-3 fatty acid, inhibits human prostate cancer cell viability
Camden Rouben	Evaluation of the shape and depth of the collateral groove of the foot as a method to predict the position of the distal phalanx within the hoof capsule
Daniela Pennington	Role of Adiponectin in Niacin-Mediated Reduction in Hepatic Triglyceride Storage
Elizabeth Ortiz	Pregnenolone 16-alpha Carbonitrile Impairs Testosterone Biosynthesis in Rat Leydig Cells
Erin Aufox	Analysis of two formulations of ceftiofur (Naxcel® and Excede®) in the seminal plasma of normal stallions
Farah Ahraf	Stearidonic Acid Sensitizes Human and Canine B-Cell Lymphoma Cells to Vincristine and Cyclophosphamide
Hannah Findlay	Testing a Synthetic Antioxidant on a Mitochondrial Toxin in NIH/3T3 Mouse Fibroblast Cells
Leah Guidry	Correlation between examination and Mimics 3-dimensional reconstruction of the equine heel
Matt Boothe	Elevated prolactin levels increase neurogenesis in the male rat
Kasandra Morelock	A Regression Analysis Study for Prediction from Colic in the Horse
Nicole McAdams	Use of an Adenoviral Vector to Deliver PNP to Canine Melanoma and Mammary Gland Tumor Cells
Tiffany Peterson	Examination of Biomarkers of Neurodegeneration in Feline GM2 Gangliosidosis

Graduate Students

Allison Bradbury	Role of Microglia Cells in the Pathogenesis of Feline GM2 Gangliosidosis
Caterina Lazzaroni	Fluroquinolone-induced mutations in SoxS, a regulator of AraC/XylS efflux pump activity
Farruk Kabir	Novel genetic defects in p16/INK4A and differential expression profile of CKI tumor suppressors in canine melanoma and mammary tumor models
Yewande Fasina	Flow Cytometric Analysis of Proliferative Responses of Chicken Peripheral Blood Mononuclear Cells Following Concanavalin A Stimulation
James Gillespie	The Use of Phage Proteins Containing NGR-Motive for Targeted Delivery of Liposomal Doxorubicin (LipoDox) into Lung Cancer Cells

Graduate Students (continued)

Joanna Hyland	Oral Bacteriophage Treatment to Reduce Fecal Shedding of *Salmonella enterica* Serotype Newport from Calves
Madhukar Lohani	Potential anxiolytic mechanisms of *Scutellaria lateriflora* (American skullcap)
Sneha Joshi	Protein Phosphatase PPM1A Positively Regulates Human Pregnane Xenobiotic Receptor-Mediated *CYP3A4* Gene Expression in HepG2 Human Liver Hepatocarcinoma Cells
Kamoltip Thungrat	Determination of Virulence of Uropathogenic Canine *Escherichia coli*
Victoria McCurdy	Significant Therapeutic Benefit after Postsymptomatic Gene Therapy in a Feline Model of Sandhoff Disease

Undergraduate Students

Jeffrey Haney	Towards developing a PNP Activated Therapy for Canine Cancer
Amanda Martin	Evaluation of *sdiA* Expression in *Escherichia Coli* on Antimicrobial Resistance
Kaitlyn Caraway	Precision of the Neubauer Hemocytometer in Quantifying Spermatozoal Concentration: Does the Experience Level of the Operator Affect Results?
Cliff Deerman	Lung Cancer Specific Phage Fusion Protein Modulates the Cytotoxicity of Liposomal Doxorubicin (LipoDox)
Hannah Young	The Variation of Articular Cartilage Stiffness among Diverse Equine Joint Types
James Diskin	Liposomal Doxorubicin Targeted by Fusion Phage Protein
Kimberly Roberts	Identification of cell compartment-specific gene expression events associated with estrogen receptor-alpha dependent endometrial development using multispectral imaging and digital image processing
Mary Herrick	Diagnostic PCR-based Assay for Feline GM2 Gangliosidosis
Robert Johnson	IBV- Ab and S1 Spike Protein Dominant B cell Epitopes Induced After Ocular Immunization with Ad5-S1
Grace Thaxton	Comparison of complete genome sequences of ArkDPI-derived infectious bronchitis virus vaccine before and after a single passage in chickens

Post-graduate / Faculty

Barbara Kemppainen	Evaluation of digoxin as an internal standard for analysis of bioactive compound [astragaloside IV] in medicinal plant *A. membranaceus* using High Performance Liquid Chromatography-Evaporative Light-Scattering Detection (HPLC-ELSD)
Heather Gray-Edwards	MRI and MRS of AAV treated feline and ovine models of Gm2 gangliosidosis
Marrilee Holland	Transabdominal Ultrasound of Adrenal Glands in Horses
Kodeeswaran Parameshwaran	Adverse effects of rotenone-induced mitochondrial complex I inhibition in mouse hippocampus are reversed with antioxidant treatment

Graduate Student Platform Presentations

Efficacy of the Combination of Mechlorethamine, Vincristine, Melphalan, and Prednisone (MOMP) for Rescue Chemotherapy in Dogs with Lymphoma: 99 Cases (2005-2012)

Amy R. Back[1], Stephanie Schleis[1], Olya Smrkovski[2], Ji-In Lee[2], Annette Smith[1], Jeffery Phillips[3]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL

[2]Department of Clinical Sciences, College of Veterinary Medicine, University of Tennessee, TN

[3]Animal Emergency Critical Care and Referral Center, Knoxville,

Introduction. The treatment of relapsed canine lymphoma can be difficult. Oftentimes, MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) is used as a rescue protocol. Procarbazine may not be economically feasible for some owners; therefore, MOMP was evaluated.

Methods. Records from Auburn University College of Veterinary Medicine and the University of Tennessee College of Veterinary Medicine were reviewed for cases of relapsed canine lymphoma treated with MOMP. Mechlorethamine (3mg/m2) and vincristine (0.75mg/m2) were administered on days 0 and 7 with melphalan (2mg/m2) and prednisone (40mg/m2) administered on days 0-13, of a 28-day cycle. WHO response criteria, response rate (RR), toxicity, and time to progression (TTP) estimates via Kaplan-Meier were evaluated. Toxicity was graded using the VCOG-CTCAE.

Results. Ninety-nine dogs were identified. Dogs were classified as follows: stage III (n=27), IV (n=32), V (n=25), substage a (n=60), and b (n=24). Dogs received a median of four drugs (range 3-9) for a median of 146 days (range 30-973) before MOMP. Dogs received a median of one rescue protocol before MOMP (range 0-4). In total, 208 cycles were administered. The RR was 48.4% (11 CR, 37 PR) for a median of 28 days (range 1-1151). As the first rescue protocol, the RR was 64.2% (7 CR, 19 PR) for a median of 43 days (range 0-858), p = 0.265. Fifty-one percent experienced gastrointestinal and/or hematologic toxicity (41% grade I).

Conclusions. The MOMP seems well tolerated and is an option for relapsed canine lymphoma. Clinically, MOMP may be best utilized as the first rescue protocol.

Acknowledgments. I would like to thank Ji-In Lee, Dr. Olya Smrkovski, and Dr. Jeffery Phillips for their contribution of cases. I would like to thank Drs. Schleis and Smith for helping with the data interpretation and statistics.

Beta-Defensin 1, 3, 102, 103, 108, 119, 120, 122 and 123 RNA Expression in Canine Oronasal Tissues

Michelle S Aono[1], John C Dennis[1], and Edward E Morrison[1]

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Introduction
Defensins are endogenous antibiotics that show tissues specific expression and have varying degrees of antibacterial, antiviral, antifungal and antiparasitic activity. Differential expression of beta-defensins in conjunction with other antibiotic agents is thought to contribute to local susceptibility. Little is currently known about beta-defensin expression in the nose, so five sites within the canine nasal cavity were surveyed for RNA expression of nine beta-defensins.

Methods
Tissues collected included olfactory bulb (brain), tongue, and five locations in the nasal cavity from dogs that were euthanized for reasons unrelated to this project. Trizol isolated total RNA diluted to 100 ng/ul was used in iScript One-Step RT-PCR with SYBR Green (Biorad) assays with primers for canine beta-defensin 1 (cBD1), 3, 102, 103, 108, 119, 120, 122, 123, and reference gene RPS5. PCR products were run on 2% agarose gels and imaged using a BioDoc-It imaging system (UVP).

Results
cBD1 shows constitutive RNA expression in all tissues tested. cBD108 RNA is expressed in the respiratory epithelia and olfactory bulb. cBD119 and 123 show low levels of expression in parts of the respiratory, olfactory epithelia and higher expression in the olfactory bulb. cBD103 is expressed in the alar fold and tongue. cBD3, 102, 120, 122 do not show appreciable expression in the tissues tested.

Conclusions
cBD1 shows constitutive expression in all tissues tested which, taken together with previous reports (Leonard et al., 2011. J. Innate Immunity 4:248), indicates it is present throughout respiratory tract, oral cavity and brain. cBD108 was localized to the respiratory epithelia and olfactory bulb, indicating it may play a role in defending olfactory neural tissue from pathogens. The cBD119 and 123 expression in the olfactory bulb indicates a role in the innate immune system of the central nervous system. cBD103 shows expression in areas where stratified squamous epithelia is present.

Acknowledgments
Financial support came from Grant #CA 01-G-022 DHS (EEM), the U.S. Department of Homeland Security, Science & Technology Directorate and Auburn University College of Veterinary Medicine 2012 Animal Health & Disease Research Grant.

Is Extra-Label Drug Use of Previcox® in Equine Patients Safe and Effective?

Heather A. Davis[1], Jennifer Taintor[2], Crisanta Espindola[1], Jameson Sofge[1], Dawn Merton Boothe[1]

[1]Department of Anatomy, Physiology, and Pharmacology, Auburn University, AL

[2]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL

Introduction. Firocoxib is a second generation selective COX-2 inhibitor approved for use in horses (Equioxx® paste) and dogs (Previcox® chew). Equine practitioners are substituting Previcox® for Equioxx® because it is cheaper. However, scientific support for this substitution is absent. Yet, the narrow therapeutic window which characterizes use of all NSAIDs mandates that extrapolation of dosing regimens –including drug products – be substantiated through appropriate clinical trials. The purpose of this study is to determine, in horses, whether or not firocoxib as Previcox® is orally bioavailable, achieves therapeutic concentrations, and thus is presumably safe for use in equine patients. The purpose of this study is to compare in adult, healthy horses, the oral bioavailability of Equioxx® to Previcox®.

Methods. Using a randomized double cross-over clinical trial design, adult horses (n=8) received a single loading dose of firocoxib as an oral (PO) paste (0.3 mg/kg), chew (0.3 mg/kg), or intravenous solution (IV) (0.2 mg/kg). Chews were administered by hand feeding; if necessary, the chew was crushed, mixed with water and administered in a syringe. Plasma samples were collected intermittently for at least 3 drug half-lives (96 hr). After a washout period, animals were rotated to their next treatment preparation. Firocoxib was quantitated in plasma by high performance liquid chromatography; the assay was validated in horses (LOQ 25 to 2500 ng/ml). Concentration versus time data was subjected to non-compartmental analysis (WinNonLin®). To assess safety, serum chemistry profiles were performed after the last trial in each animal.

Results. All animals tolerated all doses with no apparent adverse events. Animals willingly ate the chews with one exception; in contrast, all animals resisted treatment with the paste. Comparison of pertinent pharmacokinetic data revealed: C_{max} (ng/ml); 143.2 ± 31 (paste) vs. 134.5 ± 25 (chew); elimination half-life (h) of 59 ± 27 (paste), 49.2 ±15 (chew); and area under the curve (AUC) (ng/ml/hr): 8942.8 ± 3314.5 (paste) vs. 8712.4 ± 3625.5 (chew). The absolute of Previcox® bioavailability (F) was 74% (paste) vs. 72% (chew). Relative bioavailability for Previcox® vs. Equioxx® was 97.4%, indicating unity of absorption between the two products.

Conclusions. This study suggests that Previcox® is potentially equal to Equioxx® paste in terms of oral bioavailability and as such, does not present a danger in safety or efficacy if substituted for Equioxx®.

Biomechanical Testing of a Novel Tendon Implant Device for the Repair of Equine Flexor Tendon Lacerations

Elizabeth J. Barrett[1], Amelia S. Munsterman[1], and R. Reid Hanson[1]

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Introduction. Flexor tendon lacerations in horses are a serious injury that results in loss of the biomechanical function of the tendon and support of the weight bearing column. An ideal tendon repair would not compromise blood flow to the healing tendon, would allow immediate return to full weight bearing and would minimize adhesion and scar formation during healing. Current repair techniques fall short of each of these goals. A stainless steel suture device (Teno Fix Tendon Repair System, Ortheon Medical, Columbus, OH) is well established in human orthopedic surgery practice. It consists of two stainless steel anchors placed within the tendon on either side of the laceration. A piece of stainless steel suture is then fed through the anchors and the tendons are pulled together under slight tension, 1N. A steel bead is then crimped onto the suture to lock it in place and the epitenon is closed over the anchors and around the laceration. Studies in dogs and humans have shown the device is well tolerated, producing a healed tendon with minimal scarring when compared histologically to a commonly used sutured method. Unlike previous laceration repair methods, this system uses an anchor to lodge a linear stainless steel suture completely within either end of the severed tendon, so no portion of the suture is located outside of the tendon. The goal of our study was to compare the strength of this stainless steel suture tenorrhaphy technique to the currently recommended three-loop pulley pattern, in an *in vitro* model for equine tendon laceration repair.

Methods. – One tendon of each of 8 pairs of SDFTs was randomly selected to be repaired with either the three-loop pulley (3LP) suture pattern or with four stainless steel suture and anchor implants (SA). Ultimate load to failure, mode of failure, gap at failure, and load to create a 2 mm gap were obtained using a materials testing system synchronized with a high speed camera. Statistical evaluation was made using a Student's T-test, with significance set at $P \leq 0.05$.

Results. The 3 LP failed at a significantly (P = 0.0001) greater load than SA, but the load to a 2 mm gap was not significantly different. Mode of failure was by suture pull out and anchor pull out respectively. The gap at failure was significantly larger in the 3LP, than in the SA repair (P = 0. 000005).

Conclusions. Load to 2 mm gap formation is a clinically significant test, because gaps larger than 2 mm in lacerated tendons produce a weaker tendon callous than gaps smaller than 2 mm. Both the SA repair and the 3LP resisted similar strength to 2 mm gap formation.

Acknowledgments. Funding for this study was provided by the Birmingham Racing Commission and by Ortheon Medical. We wish to acknowledge Dr. Ray Wilhite for his artistic contributions, and Dr. Rob Gillette for his assistance with developing the project design.

Functional Study of the Cytoplasmic End of Transmembrane Domain 3 and Intracellular Loop 2 of Human Melanocortin-3 Receptor

Hui Huang[1], Shu-Xiu Wang[1], and Ya-Xiong Tao[1]

[1]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL, USA

Introduction. The melanocortin-3 receptor (MC3R) is a G protein-coupled receptor (GPCR) primarily expressed in the central nervous system. It plays an important role in regulating feed efficiency and nutrient partitioning, and it also modulates the expression of rhythms that anticipate nutrient availability. MC3R couples to Gs, and stimulates adenylyl cyclase to produce cAMP. Previous studies reported conflicting data on whether activation of MC3R stimulates ERK1/2 phosphorylation or not. Intracellular loop 2 (IL2) has been reported to be important for receptor function in other GPCRs. To gain a better understanding of the structure-function relationship of the MC3R, we performed alanine scanning mutagenesis to determine the function of each residue in IL2 as well as the highly conserved DRYxxI motif at the end of transmembrane domain 3.

Methods. Human MC3R mutants were generated by QuikChange site-directed mutagenesis kit. Then WT and mutant receptors were transiently expressed in HEK293T cells by calcium phosphate precipitation method. Ligand binding assays were performed on intact cells using ^{125}I-NDP-MSH with or without different concentrations of unlabeled NDP-MSH or α-MSH. Concentrations of intracellularly accumulated cAMP were measured by radioimmunoassay. Cell surface expression levels of these mutants were quantified by flow cytometry. The levels of ERK1/2 phosphorylation were measured by western blot.

Results. Of the eighteen mutants, no mutant had decreased cell surface expression. Ten mutants (D178A, R179A, Y180A, I183A, Y185A, R188A, Y189A, I192A, M193A, and T194A) had decreased maximal binding and signaling. Three mutants (V181A, T182A, and L187A) with relatively normal ligand binding were defective in signaling. L187A also had decreased basal cAMP production. Stimulation of wild type hMC3R by NDP-MSH stimulated ERK1/2 phosphorylation. Five mutants (Y185A, A186G, R188A, M193A, and T194A) did not respond to NDP-MSH stimulation with increased ERK1/2 phosphorylation; these mutants also had decreased maximal cAMP production. The other eleven mutants had increased ERK1/2 phosphorylation when stimulated by NDP-MSH.

Conclusions. These data provided comprehensive information on the structure-function relationship of the IL2 and cytoplasmic end of the third transmembrane domain of MC3R. We confirmed that hMC3R activated MAPK pathway. We identified residues that were critical for ligand binding, signaling in Gs-cAMP-PKA pathway, and signaling in MAPK pathway. Eight mutants that were defective in cAMP pathway (D178A, R179A, Y180A, V181A, T182A, I183A, L187A, I192A) had normal response in ERK1/2 pathway, demonstrating biased signaling.

Acknowledgments. This study was supported by grants from the National Institutes of Health R15DK077213 and Animal Health and Disease Research Program of Auburn University College of Veterinary Medicine.

Evaluation of Intra-abdominal pressure in Cribbing Horses

Valeria Albanese[1], Amelia S. Munsterman[1], Reid Hanson[1] and Fred DeGraves[2]

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[2]Department of Agriculture, Western Kentucky University, Bowling Green, KY

Introduction. The stereotypical behavior of cribbing has been associated with an increased incidence of small intestinal strangulation through the epiploic foramen. The effect of cribbing behavior on intra-abdominal pressure (IAP) has not been directly assessed. Based on clinical observations of increased IAP during coughing, urination and defecation, it is likely that cribbing causes an increase in IAP. Therefore, the goal of this study was to measure the IAP of horses before, during and after the cribbing behavior. We hypothesized that IAP of horses at the time of cribbing would be significantly higher compared to resting IAP and to horses that do not crib.

Methods. A prospective cohort study was designed that included 8 healthy cribbing horses (cribbing cohort) and 8 healthy non-cribbing horses (non-cribbing cohort). Food was withheld for 24 hours and water for 3 hours prior to instrumentation. A microsensor catheter was introduced into the peritoneal cavity through the right flank, using local anesthesia, for measurement of IAP. Baseline IAPs were obtained over a 30 minute period. At 30 minutes, a wooden board was positioned adjacent to the horse's head, to elicit cribbing behavior in horses from the cribbing cohort. At 90 minutes, the wooden boards were removed from those horses and IAP monitored and recorded for an additional 30 minutes. All episodes of cribbing were recorded and counted. Horses in the non-cribbing cohort were evaluated similarly, except that a wooden board was not provided. At the end of the 120 minutes, the intraperitoneal catheters were then removed and an analgesic was applied to the insertion site. The horses were turned loose in their stalls, provided feed and water, and monitored for an additional 48 hours for colic, altered vital parameters, and complications such as swelling, pain and heat at the sites of instrumentation. IAPs of cribbing horses were compared to the non-cribbing cohort.

Results. Baseline IAPs were not significantly different between cribbing and non-cribbing cohorts ($P=0.0764$). However, IAPs in the cribbing cohort were significantly increased when compared to the non-cribbing cohort, during the period of active cribbing behavior ($P=0.0016$). The number of cribbing incidents in horses that crib was not associated with the increase in IAPs ($P=0.347$). IAPs in the cribbing cohort remained significantly elevated compared to the non-cribbing cohort, even after the behavior had ceased ($P=0.0002$).

Conclusions. Cribbing is associated with increased IAP in the horse, both during and after the behavior. Cribbing has previously been associated with an increased incidence of small intestinal strangulation through the epiploic foramen. Increases in IAP associated with cribbing, as observed in this study, offer a potential explanation for the development of epiploic foramen entrapment and warrant additional study.

Acknowledgments. The authors would like to thank Dr. McCall for her help with the cribbing horses, Dr. Taintor for her help with the teaching horses and ACVS Foundation Surgeon-in-training Research Grant program for funding the research.

Synthetic Peptide Antigens for Molecular Serology of *Chlamydia* species

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[2]Federal Research Institute for Animal Health, Jena, Germany

Introduction. The obligate intracellular bacterial genus *Chlamydia* includes nine human and animal pathogenic species. Serological diagnosis of chlamydial infection such as complement fixation or microimmunofluorescence tests has low sensitivity and is technically challenging, difficult to standardize, requires cumbersome production of antigens, and demands skilled technicians. Moreover, all chlamydial species are closely related, and serological assays use antigens such as highly cross-reactive whole chlamydial organisms, highly conserved proteins, or the genus-specific LPS. This high degree of serological cross-reactivity prevents conclusive species-specific serology. Thus, simple ELISA tests that differentiate the species reactivity of antichlamydial antibodies with high specificity and sensitivity are urgently needed.

Methods. B cell epitopes that are present only in a single chlamydial species were identified within the complete proteomes of all nine chlamydial species. Unique proteins or unique continuous peptide epitopes were ranked based on published immunodominance of the proteins and on polymorphisms in the protein alignment of all nine species. Potential linear (continuous) B-cell epitopes from high ranked unique peptide-regions were predicted *in silico* based on physiochemical properties such as hydrophilicity, flexibility, turns, and solvent accessibility by the BCPRED algorithm. High scoring peptides are selected and synthesized chemically with an N-terminal biotin followed by a serine-glycine-serine-glycine spacer and the specific amino acid sequence (8-20-mer). Streptavidin-coated microtiter plates, horseradish peroxidase conjugated anti-mouse Ig and chemiluminescent substrate were used in an ELISA format to test reactivity of each peptides with each of nine *Chlamydia* species mono-specific hyperimmune sera raised in A/J or Balb/c mouse strains. Reactive peptides were ranked based on species specificity and signal magnitude they produced in ELISA format.

Results. We have identified 3-10 species-specific peptides each for *C. pneumoniae*, *C. pecorum*, *C. abortus*, *C. psittaci*, *C. trachomatis*, *C. muridarum*, *C. caviae*, *C. suis* from proteins such as OmpA, Omp2, PmpD, IncA, IncG, CT442, IncCT529, IncCT618, and TarP. Also, we have identified several *Chlamydia* genus-specific peptides that have potential to identify all chlamydial species, as well as strain-specific peptides for *C. pecorum*, *C. trachomatis*, and *C. suis*.

Conclusions. Our peptide antigens produce high and absolutely species-specific signals in a robust ELISA format. Currently, *C. felis*-specific sera and peptides are produced to complete the project. These peptides will also be used in peptide microarray technology for detecting antibodies against *Chlamydia* species. We anticipate that *Chlamydia* species-specific peptide ELISAs will improve serodiagnosis of *Chlamydia* species infections, and further understanding of chlamydial pathogenesis in retrospective studies of animal and human chlamydial infections and in testing of vaccine efficacy in epidemiological investigations.

Acknowledgments. We thank Dongya Gao for help with chlamydial PCR typing, and Garry Myers for pre-publication access to the *C. suis* genome.

Culture and Characterization of Late Outgrowth Endothelial Colony Forming Cells in Adult Horses

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[2]Department of Chemical Engineering Samuel Ginn College of Engineering, Auburn University, AL

Introduction. Endothelial progenitor cells (EPCs) are derived from bone-marrow stem cells, circulate in peripheral blood, and function in vascular homeostasis and repair. Equine EPCs have yet to be investigated in horses and could benefit the equine regenerative therapy field for treatment of diseases with poor vascularization such as fractures, ischemia, non-healing wounds and chronic laminitis. Horses with Equine Metabolic Syndrome could have lower numbers of functional EPCs than normal horses. There is evidence in human research that decreases in EPCs can be markers for cardiovascular disease, metabolic disease, obesity, and diabetes and that the functional properties of these cells are reduced. The purpose of this study was to culture a subgroup of EPCs, late outgrowth endothelial colony forming cells (LOCs), from healthy horses. Cultured equine cells were characterized as true LOCs using phenotypic assays of vascular tube formation in Matrigel© and uptake of acetylated low density lipoprotein (Di-Ac-LDL).

Methods. A 5 mL heparinized blood sample from a quarter-horse gelding was cultured with supplemented endothelial growth medium. After 24 hours of incubation (37°C, 5% CO_2 and 95% humidity), the blood media mixture was removed and replaced with 20 mL of fresh medium, placed into the incubator and observed daily for colony formation. Once colonies formed, they were counted, harvested, expanded, and used for characterization assays. Vascular tube formation in Matrigel was assessed after 5 and 24 hours in culture, and vascular tubes recorded using light microscopy. For the Di-Ac-LDL uptake assay, cells were cultured with fluorescent labeled Di-Ac-LDL solution for 6 hours. Cells were counterstained with 4',6-diamidino-2-phenylindole and imaged with a fluorescent microscope. Human LOCs served as a positive control and 3t3 fibroblast cells served as the negative control for both assays.

Results. There were 18 colonies per 5 mL of blood seen 10 days post initiation of cell isolation. Cells had characteristic single layer cobblestone morphology and significant outgrowth upon expansion. Equine and human LOCs formed vascular tubes in Matrigel. Equine and human LOCs showed an uptake of Di-Ac-LDL, (90% positive cells). 3t3 fibroblast cells did not uptake any Di-Ac-LDL or form vascular tubes.

Conclusions. Based on the results from this study, LOCs can be isolated and cultured from peripheral blood samples of healthy horses. This provides information for future research investigating additional characterization methods and possible therapeutic use of LOCs and EPCs in horses with diseases that alter vascular function.

Acknowledgments. The authors thank Qiao Zhong and William Batchelor for technical assistance. This study was supported by funding from the Department of Chemical Engineering and the Department of Clinical Sciences.

ORAL FIBROSARCOMA IN THE CAT: 37 CASES (1998-2011)

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[4]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, NY

[5]The Veterinary Cancer Society, Norwalk, CT

Introduction. Fibrosarcoma (FSA) is the second most common oral tumor in cats. There is little information regarding the presentation, optimal treatment, and prognosis in cats with oral FSA. The goal of this study was to describe the biological behavior of feline oral FSA and to determine what factors impacted outcome.

Methods. A multi-institutional retrospective study of cats diagnosed with oral FSA between 1998 and 2011 was performed. Data on signalment, history, clinical signs, diagnostics, treatments, and response to treatments was reviewed.

Results. Thirty-seven cats with a histological diagnosis of FSA arising from the oral cavity were included. The median age at initial presentation was 12 years (range: 3.1 – 17.7). Thirteen of 32 (40.6%) cats were treated with surgical excision alone, 10 (31.3%) cats with surgery radiation therapy (RT) and/or chemotherapy (CTH), and nine (28.1%) cats with RT or CTH alone. Twenty-one (65.6%) cats developed local recurrence after treatment and one (3%) developed known metastatic disease. Treatment (190 days vs. 33 days, $p = <0.001$) and incidentally found tumors (2,227 days vs. 111 days, $p = 0.033$) were associated with longer median survival times (MST). Tumor location, treatment modality, age, sex, stage and tumor grade were not significantly associated with MST.

Conclusions. Results of this study suggest that feline oral FSA affects older cats and is associated with a poor prognosis due to lack of local disease control. Treatment and the absence of clinical signs at presentation are associated with longer survival times.

Acknowledgments. None.

Role of Microglia Cells in the Pathogenesis of Feline GM2 Gangliosidosis

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Introduction. Microglia cells are the primary resident immune cells of the CNS. Non-activated, resting microglia persist with a ramified morphology, consisting of a small, round cell body with long, branching processes used to survey for bacteria, viruses, cell debris, or degenerating neurons. Once activated, microglia assume an ameboid morphology capable of phagocytosis, express MHCII to present antigens and activate T-cells, and secrete pro-inflammatory molecules. A well established feline model of GM2 gangliosidosis (GM2) provides a means to further define the role of microglia in neurodegeneration. Additionally, therapeutic intervention with an AAV vector encoding the deficient enzyme, Hexosaminidase A, (AAV-Hex), has proven to significantly delay disease onset, and thus should curtail the activation of microglia.

Methods. Four-week old cats affected with GM2 gangliosidosis were treated by bilateral thalamic and deep cerebellar nuclei (DCN) injections of AAV-Hex. Three cats were euthanized at 16 weeks post-treatment for short term analysis while three remaining cats were followed to humane endpoint. For immunohistochemistry, 6µm paraffin-embedded sections were blocked for 1hr with 5% normal horse serum, treated for 1hr with mouse anti-feline MHCII (1:4) or rabbit anti-human/rat/mouse Iba1 (1:100) followed by biotin-labeled horse anti-rabbit/mouse IgG for 1hr, ABC Reagent for 30 min, and visualized with DAB substrate.

Results. Untreated GM2 cats exhibited an overwhelming population of activated microglia characterized by ameboid morphology and expression of MHCII and Iba1 (a marker of both resting and activated microglia). In comparison, a normal, age-matched control displayed non-activated microglia of the ramified nature and lacked positive staining for MHCII. These results were consistent throughout the cerebrum, cerebellum, and spinal cord. Three GM2 cats treated with thalamic and DCN injections of AAV-Hex and euthanized 16 weeks post-treatment, with negligible indication of disease, displayed histological features of microglia indistinguishable from normal cats. One cat euthanized at 10 months of age due to non-neurologic reasons had a population of microglia almost entirely of the resting, ramified nature consistent with successful delay of disease onset. However, two cats treated for long-term evaluation (>1yr), with pronounced symptoms of disease, revealed activated microglia typical of late disease stages. Additionally, in one cat treated in the thalamus but not the DCN, resting microglia predominated in the cerebrum, while activated microglia were readily apparent in the cerebellum.

Conclusions. Excess ganglioside in the neurons of GM2 cats leads to activation of microglia, characterized by ameboid morphology and expression of MHCII. However, AAV mediated gene delivery delays disease onset and activation of microglia. Chronically activated microglia present during late stages of disease likely contribute to inflammation, cytotoxicity, and accelerate disease progression.

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Resistance of Jax® A/J mice against chlamydial lung infection mediated by asymptomatic *Klebsiella* spp. respiratory infection.

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Introduction: The A/J mouse strain has been for some time preferred for the murine model of lung infection by *Chlamydia* spp. bacteria. Due to the low innate inflammatory response, A/J mice previously not exposed to chlamydiae ("naïve") respond with low elimination of the bacteria in the first week after intranasal challenge inoculation and develop severe lung disease characterized by interstitial mononuclear infiltrates and increased lung weight. If a protective adaptive Th1 cell response emerges after the first week, A/J mice quickly eliminate chlamydiae. Therefore, A/J mice that have been pre-exposed to a low-dose chlamydial challenge ("live-vaccine") are highly protected against high-dose chlamydial challenge and eliminate the chlamydiae efficiently without disease. For this reason, the A/J strain is essential for chlamydial vaccine discovery and development. Unexpectedly, A/J mice from The Jackson Laboratory showed a fully resistant behavior against a high inoculum (10^8 elementary bodies) of the highly virulent *Chlamydia abortus* strain. Here, we describe analyses that identified the reason for this unexpected resistance.

Methods: Inbred A/J female mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 5 weeks of age. *Chlamydia abortus* strain B577 (ATCC VR-656) was used in all experiments. Live-vaccine mice were intranasally immunized with 1×10^6 genomes of viable *C. abortus,* and 4 weeks later both live-vaccine as well as naïve groups were challenged intranasally with 1×10^8 genomes of *C. abortus.* Ten days post infection mice were sacrificed and changes in body and lung weight were determined. *C. abortus* lung loads were determined by FRET real-time PCR.

Results: To eliminate the possibility of inoculum degradation, the *C. abortus* B577 stock was genetically re-typed and quantified by FRET real-time PCR. Since these parameters were unchanged, we investigated the effect of different inoculum doses in naïve A/J mice, and found a completely resistant phenotype at all dose ranges. To test for possible genetic drift in these mice or the effect of breeding facility-specific, we tested mice from two different Jackson facilities (AX9 and MP16) and found again a fully resistant phenotype. Analysis of husbandry records revealed that mice in the standard barrier facilities were affected by asymptomatic respiratory infection with *Klebsiella* spp. In contrast, A/J mice from the *Klebsiella* spp.-free maximum barrier facility, MP14, showed a fully susceptible phenotype. We confirmed the resistance-inducing effect of the *Klebsiella* spp. infection by reversal of the resistant to the susceptible phenotype by an antibiotic treatment of the mice prior to *C. abortus* challenge.

Conclusions: Collectively, these data strongly suggested that the asymptomatic *Klebsiella* spp. infection in the Jax® A/J mice caused their resistant phenotype, possibly by acting as a biological response modifier that activated the innate immunity at the respiratory mucosal epithelia.

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Mutational analysis and factor VIII sequence in a colt with hemophilia A

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Introduction: Hemophilia A is an X-linked disorder caused by a deficiency in factor VIII (FVIII) and is the most common inherited coagulation disorder in horses. Hemophilia A has been linked with mutations in the FVIII gene, promoter, and von Willebrand factor (vWF) binding site. Combined deficiency of factor V and FVIII is an autosomal recessive coagulation disorder identified by mutations in either LMAN-1 (lectin mannose-binding 1) or MCFD2 (multiple coagulation factor deficiency 2). Although foals have been appropriately diagnosed with a coagulopathy based on deficiency in FVIII activity, the causative gene mutations in horses have not been identified. Distinguishing between an autosomal recessive disorder, maternal inheritance and a spontaneous mutation is essential for future breeding. The objective of this project was to determine the coding sequence of FVIII in the horse and identify the causative mutation in a colt with hemophilia A.

Methods: Genomic DNA was isolated from EDTA whole blood in a normal horse, the affected colt, and the colt's dam utilizing a commercial kit (Qiagen). Polymerase chain reactions (PCR) were performed with primers designed from predicted sequence for equine FVIII, FVIII promoter, vWF FVIII binding site, LMAN-1 and MCFD2. Sequence data was compared for identification of mutations and maternal inheritance. To evaluate gene expression of FVIII, hepatic mRNA was isolated from a necropsy specimen from the affected colt and a normal horse using a commercial kit (Qiagen). Reverse transcription PCR was used to create first strand cDNA from RNA template. PCR reactions were performed on the cDNA with primers designed across the FVIII coding sequence. Protein expression of FVIII in the affected colt versus the normal horse was performed via Western immunoblot on hepatic lysate.

Results: Genomic sequence for the FVIII gene (exons and adjacent splice sites), FVIII promoter, LMAN-1, and MCFD2 were identical in all animals. A single nucleotide polymorphism (A>C) was detected in exon 22 of the vWF FVIII binding site in the mare and the colt, leading to an amino acid change from lysine to asparagine. The mutation was identified in samples from 3 normal horses, suggesting the mutation did not significantly affect the binding site. PCR for FVIII hepatic cDNA revealed normal product for the foal and unaffected horse from exons 2-26. A product was not obtained across exons 1-2 in the foal. Western immunoblot revealed normal sized FVIII protein in the normal horse, but a product was not obtained for the foal.

Conclusions: Although genomic sequence did not identify a mutation in the coding sequence of the FVIII gene, the lack of PCR product for the foal across exons 1-2 in the cDNA suggests a mutation in the non-coding portion of FVIII (specifically intron 1). Western immunoblot further supported that the foal was not producing a normal sized product for FVIII. Genomic evaluation of intron 1 in FVIII is warranted.

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Disposition of Levetiracetam in Healthy Adult Horses

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Introduction. Seizures in horses have equine welfare and human safety implications. Conventional antiseizure therapy for horses is outdated when compared to recent advances in human, canine and feline medicine. Levetiracetam (LEV: Keppra®) is a new, safe, and efficacious antiepileptic drug (AED) with a unique mechanism of action, but pharmacokinetic studies in horses are lacking. The objective of this project was to determine appropriate dosing of LEV in horses. LEV will likely provide improved treatment of seizures in horses.

Methods. Eight horses received 20mg/kg of intravenous LEV ($_{IV}$LEV), 30mg/kg of oral, immediate release LEV (LEV_{IR}), and 30 mg/kg of oral, extended release (LEV_{ER}) in a 3-way randomized crossover design, with > 7-days washout period. Serum samples were collected over 48 hours, and LEV concentrations determined by immunoassay.

Results. Maximum mean ± standard deviation serum concentrations after administration of LEV_{IR} and LEV_{ER} were 50.7 ± 10.6 μg/mL and 53.6 ± 15.9 μg/mL, respectively. For $_{IV}$LEV, LEV_{IR} and LEV_{ER} half-life and area under the curve by dose were 5.2 ± 1.35 hours, 7.06 ± 1.93 hours, 6.38 ± 1.98 hours and 856 ± 189 min*kg*μg/mL/mg, 813 ± 140 min*kg*μg/mL/mg and 842 ± 213 min*kg*g/mL/mg, respectively. Total body clearance after IV administration and clearance by bioavailability after oral administration of LEV_{IR} and LEV_{ER} were 1.24 ± 0.32 mL/min/kg, 1.3 ± 0.24 mL/min/kg and 1.3 ± 0.34 mL/min/kg, respectively. Bioavailability of LEV_{IR} and LEV_{ER} was 96 ± 10% and 98 ± 13%, respectively. After intravenous and oral administration of LEV, concentrations achieved minimum therapeutic ranges for humans (> 5 μg/ml) for at least 12 hours and 18 hours, respectively.

Conclusions. Oral administration of 30 mg/kg of LEV_{IR} or LEV_{ER} in healthy adult horses is likely to achieve therapeutic concentrations for at least 18 hours.

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Asymptomatic endemic *Chlamydia pecorum* infections reduce growth rates in calves by up to 48 percent

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Introduction. Obligate intracellular bacteria of the phylum *Chlamydiae* infect virtually every eukaryotic organism. In cattle, the two species, *C. abortus* and *C. pecorum*, are routinely detected in acute infections with distinct clinical symptoms such as fertility disorders and abortion, mastitis, sporadic encephalomyelitis, kerato-conjunctivitis, pneumonia, enteritis and polyarthritis. However, besides these infrequent acute infections many more asymptomatic chlamydial infections can be detected in livestock. Little is known about the health and economic impact of such chronic infections in cattle. In this study, we investigated the health consequences of these low level natural chlamydial infections in calves.

Methods. In a prospective observational study, a cohort of 51 female Holstein and Jersey calves was examined from birth to 15 weeks of age. We evaluated in biweekly sampling the association of health, growth and development of calves with chlamydial infection and host response to infection. The plasma marker of liver health and inflammatory response; albumin, globulin, insulin like growth factor-1 (IGF-1) were analyzed and correlated with clinical appearance and growth in dependence of chlamydial infection intensity as determined by mucosal chlamydial burden or contemporaneous anti-chlamydial plasma IgM.

Results. *Chlamydia* 23S rRNA gene PCR and *ompA* genotyping identified only *C. pecorum* (strains 1710S, Maeda, and novel strain smith3v8) in conjunctival and vaginal swabs. All calves acquired the infection but remained clinically asymptomatic except occasional conjunctivitis. Although calves developed normally, the rates of body weight gain started declining from seven weeks of age. High chlamydial infection associated with reduction of body weight gains by up to 48% and increased conjunctival reddening ($P<10^{-4}$). Plasma level of albumin and IGF-1 were significantly reduced in highly infected calves while the globulin was increased ($P<0.05$). High anti-*C. pecorum* IgM associated eight weeks later with 66% increased growth ($P=0.027$).

Conclusions. Our result suggests substantial impact of asymptomatic chlamydial infection on growth of neonatal calf. The decreased plasma albumin, IGF-1 and increased globulin suggests liver injury by inflammatory mediators as mechanisms for the growth inhibition. Association of anti-*C. pecorum* IgM eight weeks later with increased growth indicates a potential for immune protection from *C. pecorum*-mediated growth depression. This study advocates the need of efficacious prophylactic vaccine against *Chlamydia* spp. infection in livestock.

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Molecular Cloning of CHO-K1 Cells Expressing Canine or Feline Gonadotropin-Releasing Hormone Receptor: A Research Tool for Development of Immunocontraceptives for Feral Dogs and Cats

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Introduction. Following binding of gonadotropin-releasing hormone (GnRH), GnRH receptor mediates secretion of luteinizing hormone and follicle-stimulating hormone, gonadotropins necessary for normal ovarian and testicular function. Because GnRH is crucial for initiating the reproductive hormonal cascade, it is targeted for development of immunocontraceptives for overpopulation control of various mammalian species including canines and felines. Although the canine GnRHR sequence was published, the feline sequence was unknown, increasing difficulty of experimental study of receptor-ligand interactions in this species. Here, we sequenced feline GnRHR and developed a research tool, cloned Chinese Hamster Ovary (CHO-K1) cells that express canine or feline GnRHR, to test the receptor-ligand binding properties in cell cultures. The use of such cells might minimize the use of animals in GnRH receptor research.

Methods. To generate canine GnRHR cells, total RNAs were extracted from flash-frozen pituitaries obtained from normal dogs. Full length GnRHR cDNA was amplified from total RNA by RT-PCR with dog-specific primers. To generate cells that express feline GnRHR, we amplified and sequenced feline GnRHR gene. First strand cDNA was synthesized in a reverse transcription reaction from total RNA extracted from frozen pituitaries obtained from normal cats. 5' and 3' ends of the gene were amplified using FirstChoice RLM-RACE Kit and sequenced. Based on obtained sequences of 5' and 3' ends, new cat-specific primers were designed to amplify the whole gene. The amplified product was sequenced using cat gene-specific primers. Canine and feline GnRHR inserts were cloned into separate Invitrogen® pcDNA3.1 expression plasmids. CHO-K1 cells were transfected with the plasmids containing the full-length canine or feline GnRHR sequence by electroporation.

Results. To obtain cells that express feline or canine GnRHR, the corresponding molecular inserts were constructed and cloned into CHO-K1 cells. In the process of cloning, canine GnRHR gene was found to have a single base change in position 577 when compared to the previously published sequence. This change translates to different amino acids in position 193 of the protein: proline in the published protein and serine in the sequence of GnRHR protein determined by us. Sequencing of GnRHR from different canine breeds verified that the observed single base change is not breed-related. The expression of feline and canine GnRH receptors in cloned CHO-K1 cells was confirmed at transcriptional as well as at protein levels.

Conclusions. In this study, feline GnRHR gene was sequenced for the first time and the sequence of canine GnRHR was corrected. We also cloned CHO-K1 cells expressing canine or feline GnRHR. Such cells can be used as a research tool for evaluating binding properties of GnRH-related agonists or antagonists and, thus, might aid in the development of GnRH-based contraceptive vaccines for cats and dogs.

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T cell responses to Ark-type IBV Vaccine in White Leghorns

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Introduction. Infectious bronchitis virus (IBV) causes huge economic losses in the poultry industry of South-Eastern United States. Despite vaccination, outbreaks of IBV are reported and are attributed to serotypic heterogeneity of the virus. Also, after Arkansas (Ark) serotype vaccination persistence of Ark serotype has been reported, indicating that other problems with these vaccines exist. Although little is known about the T cell response to IBV, it has been shown that cytotoxic T cells are protective against IBV challenge (Collisson et al, 2000). Based on the persistence of Ark IBV, a lack of protective T cell responses may be induced. Therefore, we measured the Ark-type IBV vaccine induced T cell responses in mucosal and systemic immune compartments.
Methods. For primary responses, 3 week old chickens were ocularly immunized with $3x10^6$ EID_{50} of Ark-type IBV vaccine. For secondary responses, chickens were vaccinated at 3 and 7 weeks of age with Ark-type IBV vaccine. CALT (Conjunctiva-associated lymphoid tissue), spleen and HG (Harderian gland) were collected from euthanized chickens and the isolated lymphocytes were counted using the tryphan blue exclusion method. In addition, total RNA was extracted from these tissues to measure IFN-γ, granzyme A and perforin expression by qRT-PCR (quantitative reverse transcriptase-polymerase chain reaction).
Results. Lymphocyte counts in secondary lymphoid tissues show a lag, expansion and contraction phase after IBV vaccination as has been reported for mammals. Primary IFN-γ expression in HG increases on day 2 post IBV vaccination, indicating an early innate NK response. The concurrent increase of granzyme A and perforin expression confirms this notion. IFN-γ expression increases significantly between days 4 and 6 in the CALT and HG in the primary response. Granzyme A and perforin expression in CALT also peak at 4 days post vaccination and remain significantly elevated until day 10. This coincides with an IFN-γ peak on day 10. An early(day2 and 3) and late (day 11) increase of granzyme A expression in the HG are followed and preceded by a IFN-γ increase in the primary response on days 5 and 10, respectively. The late increase is consistent with the observation in the literature that cytotoxic T cells are induced on day 10 of the IBV response. There is no significant increase of IFN-γ, perforin and granzyme A expression in the spleen during the primary response. After boosting no increase in expression of IFN-γ is observed in the CALT, although a significant increase in granzyme A expression is observed on days 4-7, which is consistent with an effector memory T cell response. However, an increase of IFN-γ expression is observed in the HG and spleen on day 4, which coincides with increases of granzyme A expression in spleen, and may reflect a central memory T cell differentiation and activation. There is a late increase of IFN-γ expression on day 10 in the spleen.
Conclusions. The primary IFN-γ and cell-mediated immune response to IBV occur predominantly in the mucosal immune compartment. The secondary IFN-γ response shifts from the mucosal to the systemic immune compartment, indicating the presence of a central memory T cell response to IBV. However, the cytotoxic memory T cell response was still predominantly associated with CALT. Hence, IBV vaccines induce effector memory T cell responses in CALT, but not in the systemic immune compartment, i.e., the spleen. Thus, a lack of T cell mediated immunity at mucosal surfaces is not the cause of IBV vaccine failure.
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Dramatic Phenotypic Improvement after Gene Therapy in a Feline Model of GM1 Gangliosidosis

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Introduction. Lysosomal β-galactosidase (βgal) deficiency causes neuronal storage of GM1 ganglioside, resulting in progressive neurological deterioration and death, often by age 5. AAV gene therapy has been extraordinarily successful in the GM1 mouse model, resulting in enhanced survival and complete clearance of storage in the brains of GM1 mice (*Mol Ther*, 15:30, 2007; *PLoS One*, 5:e13468, 2010). Because the mouse brain is ~1000 times smaller and much less complex than the human brain, it is important to test AAV gene therapy in an animal model whose brain size and complexity more closely resemble humans. The feline GM1 model presents an unparalleled opportunity to evaluate AAV gene therapy in a non-rodent, 'large' animal prior to initiating human clinical trials.

Methods. In the current study, AAV2/1 or AAV2/rh8 vectors expressing a feline βgal cDNA (2.9 e12 – 1.2 e13 g.c. total) were injected bilaterally into the thalamus and deep cerebellar nuclei of 2 to 3-month old GM1 cats (disease onset 4.1 ±0.5 months). A short-term cohort was euthanized 15-18 weeks post-treatment for analysis of βgal distribution. A long-term cohort was evaluated for therapeutic outcome by (1) magnetic resonance imaging (2) a clinical rating scale reflecting the neurodegenerative course of untreated GM1 cats and (3) lifespan.

Results. In treated brains collected 15-18 weeks post-injection, βgal was distributed throughout the entire anterior-posterior axis at levels between 1.3 to 3.3 times normal. The spinal cord demonstrated βgal activity 1.3 – 4.5 times normal. Activity of lysosomal hexosaminidase, which is elevated in untreated GM1 cats, was normalized in AAV-treated GM1 cats, demonstrating restoration of lysosomal function. Long-term therapeutic experiments are showing extraordinary results. Ten AAV-treated GM1 cats currently range in age from 19.0 – 37.1 months (untreated humane endpoint, 8.1 ± 0.6 months, n=14). Two cats, aged 19.8 and 29.2 months, have mild clinical signs of disease while the remaining eight cats are clinically indistinguishable from normal controls and demonstrate normalization of MRI brain architecture. Five of the ten treated GM1 cats have had mild seizures that are well-controlled by medication and presumed though not proven to result from residual brain disease not fully corrected by gene therapy. In addition, for the first time in the 40 year history of the colony, GM1 females and males have proven reproductively fertile after AAV treatment. Therapeutic benefit is also demonstrated in GM1 cats treated with a ten-fold lower vector dose (3.1 e11 g.c. total) with 2 cats currently 17.5 and 14.1 months old.

Conclusions. These translational studies provide strong support for the initiation of AAV-based clinical trials for human GM1 gangliosidosis.

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Veterinary Student Platform Presentations

Effects of intravenous lipid emulsion on blood coagulation in dogs

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Introduction: Intravenous lipid emulsion (ILE) is used in treatment of drug toxicities by facilitating removal of lipophilic drugs. In people, some studies indicate that ILE causes a decrease in platelet aggregation. Additional work suggests that ILE promotes increased coagulation in platelet poor plasma. The present study is designed to model the effect of ILE in dogs given an IV bolus of Intralipid. Thromboelastography (TEG) was used to assess clot formation in whole blood with and without addition of Intralipid. In some cases, platelet aggregation and adhesion were also assessed using the Impact-R System.

Methods: 20 healthy dogs were used in this study. Results of a complete blood cell count, chemistry panel and standard coagulation tests were normal for all animals. TEG was performed in parallel using blood with and without addition of Intralipid to yield final concentrations of 5 or 10 mg/mL. Values measured by TEG were: R (time to initial clot formation), K and α-angle (kinetics of clot formation), and MA (maximum clot strength). In some samples, cytochalasin D was used to eliminate aspects of platelet function dependent on the cytoskeleton.

Results: Average clot strength was significantly decreased in samples with Intralipid compared to baseline. There was a trend towards decreasing clot strength with increasing amounts of Intralipid, but results were not statistically significant. Clot strength was decreased in samples with cytochalasin D and Intralipid compared to cytochalasin D alone. Impact-R test results in samples with Intralipid were not interpretable due to lipid-induced artifact.

Conclusion: In vitro, Intralipid causes a change in coagulation resulting in decreased clot strength. Our results indicate that this is caused by decreased platelet contribution to clot strength but may also involve interference with other components of the clot such as fibrin crosslinking. These results may have implications for dogs with extreme hyperlipidemia.

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Impact-R Evaluation of Platelet Adhesion and Aggregation in Dogs

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Introduction. The Impact-R is a novel instrument that has been used for platelet function testing in people but with limited implementation in veterinary medicine. The instrument uses citrated whole blood to test platelet adhesion and aggregation under normal arterial flow conditions Clinical applications of the Impact-R in human medicine include evaluation of platelet function in people with inherited and acquired platelet disorders including Glanzmann thrombasthenia (GT), von Willebrand disease, and drug-induced platelet disorders. The goal of our study was to evaluate the effectiveness of the Impact-R system in detecting platelet adhesion and aggregation in normal canine blood samples.

Methods. Thirty-five canine whole blood samples were collected in tubes containing 3.2% citrate, 15% potassium EDTA, and no anticoagulant. A CBC, coagulation profile, and chemistry panel were performed to evaluate the health of each patient. EDTA platelet counts were compared to the normal canine reference interval. Citrated blood samples were analyzed by the Impact-R with and without the addition of ADP. Digital images were generated and platelet percent surface coverage (SC), aggregate size (AS), and number of objects visible (Ob) values were reported. Platelet aggregation tracings were performed on platelet rich plasma (PRP) samples from several dogs. Impact-R results were compared to the reported human Impact-R values, generated images, and platelet aggregation results. Intralipid®, an intravenous fat emulsion, was added to several samples for Impact-R evaluation. Impact-R was also performed on blood from a dog previously diagnosed with GT.

Results. Twenty-eight samples with normal platelet numbers processed without ADP generally exhibited minimal amounts of platelet adhesion and aggregation. SC and AS values from the same samples pretreated with ADP were either within limits to slightly decreased when compared to reported human reference values. Impact-R results on three samples with Intralipid® and one lipemic sample were not interpretable due to lipid induced artifacts. The GT dog sample had results similar to those seen in human GT patients.

Conclusions. The Impact-R has potential to evaluate platelet adhesion and aggregation in non-lipemic, non-thrombocytopenic canine whole blood samples pretreated with ADP. In contrast to people, canine platelets required addition of an agonist for significant activation and measurable results on the Impact-R. The GT dog results support the ability of the system to detect decreased platelet aggregation in dogs. The Impact-R system may assist in the identification of congenital or acquired canine platelet function disorders such as GT and von Willebrand disease.

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The Effects of a Synthetic Antioxidant against a Mitochondrial Toxin on NIH/3T3 Mouse Fibroblast Cells

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Introduction. Apoptosis, controlled cell death, is a natural process of development and aging. Recently it has been found that mitochondria, the "powerhouse" of the cell, play an important role in apoptosis through a signaling cascade. Recent studies found that many of the effects of aging can be replicated in cells through treatment with a mitochondrial toxin, rotenone. Rotenone is natural substance used in many pesticide products that inhibits complex I of the electron transport chain. Mouse fibroblast cells in this study were infected with rotenone and subsequently given an antioxidant as a means of recovery. A recent therapeutic option for treatment of aging complications focuses on the use of a synthetic antioxidant targeting the mitochondria. PMX-500F, a lipoylcarnitine derivative, is synthetic antioxidant that has been found to reduce reactive oxygen species (ROS) in mice. Rotenone treated cultured mouse fibroblasts in this study were co-treated with PMX-500F as a potential therapy for ROS and rotenone-induced apoptosis. Our hypothesis is as follows: Using a cell culture system, rotenone induced damage through inhibition of mitochondrial complex I and generation of ROS will be counteracted by the antioxidant, PMX-500F. Co-treatment with PMX-500F will result in reductions in:

1. ROS (as measured by DCF-DA fluorescence).
2. Apoptosis (as measured by nuclear morphology).
3. Activation of caspase-3 (as measured by western blotting).

Methods. Cells were treated with 2 µM rotenone and 2 µM PMX-500F for 24 and 48 hours. Cells were evaluated with photomicrographs, Hoechst 33342 stain, reactive oxygen species (ROS) measurement with dichlorofluorescein diacetate (DCF-DA) fluorescence, and western blotting.

Results. After 24 hour treatment, a 21.1% increase in ROS values were seen in cells with rotenone, 9.9% in cells with PMX-500F, and 15.2% in cells with both rotenone and PMX-500F. After 48 hour treatment, ROS values increased 40.7% with rotenone, 23.4% with PMX-500F, and 63.5% with both. Apoptosis in 24 hour treatments indicated an increase of 23% with rotenone, 38% with both, and a decrease of 0.5% with PMX-500F. After 48 hours, apoptosis was 36% with rotenone, 28% with both, and -3% with PMX-500F. Values represent a comparison of untreated cells. Western blotting showed the highest increase in cleaved caspase-3 in both rotenone and rotenone plus PMX-500F cells for 24 hour treatment. 48 hour treatment showed the highest increase in cleaved caspase-3 in rotenone plus PMX-500F cells.

Conclusions. PMX-500F did not reduce proapoptotic factors of treated NIH/3T3 cells to control levels. It is concluded that at a concentration of 2 µM PMX-500F and 2 µM rotenone for 24 and 48 hour treatment periods, PMX-500F did not reduce ROS levels, cleaved caspase-3 levels, or percent apoptosis in NIH/3T3 fibroblast cells. An incidental finding was that PMX-500F alone lowered ROS (as compared to rotenone), apoptotic factors, and cell overgrowth. Further studies need to be performed to definitively characterize antioxidant effects on mitochondrial function.

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The Variation of Articular Cartilage Thickness in Equine Limb Joints

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Introduction. Articular cartilage is hyaline cartilage that provides a smooth articulating surface for two bones to glide and roll past each other within a synovial joint. Changes to cartilage due to injury or pathology are a common cause of lameness in the horse. Often, a change in articular cartilage thickness is seen in association with these cases. Understanding thickness trends along with other diagnostic modalities could lead to earlier diagnoses of equine lameness and ultimately prevent the demise of articular cartilage, which lacks regenerative qualities. An overall understanding of the variations of cartilage thickness will help create a more complete knowledge of the different synovial joints in the body. With these future goals in mind, this project chose to characterize and compare the thickness of articular cartilage in three types of equine limb joints using a needle probe test technique.

Methods. The cartilage thickness of six sets of equine cadaveric joints each comprised of a fetlock, carpus, and stifle was measured using a needle probe technique. The areas of interest in the joints include the medial and lateral condyles of the distal cannon bone in the fetlock, the medial aspect of the radiocarpal and midcarpal joints in the carpus, and the medial femoral condyle of the stifle. The needle probe test was performed with a Bruker CETR UMT-3 tribometer using a 25 gauge needle on cartilage samples less than 31 hours postmortem. The integrity of fresh joints was important to mimic the cartilage conditions in vivo. Cartilage thickness was determined by comparing the displacements of the needle at the contact point of the cartilage surface to the contact point with the subchondral bone. Each sample was tested at multiple points and then statistical analysis was used to compare the cartilage thicknesses of the different joints. Further statistical comparisons were made within the surfaces of the fetlock and carpal joints.

Results. The mean cartilage thickness of the stifle was significantly greater than the thickness at both the carpus and the fetlock in all six horses analyzed ($p<0.001$). The thicknesses of the carpus and the fetlock cartilage were the same. Within the carpus, the radiocarpal joint cartilage thickness was found to be significantly greater than that of the proximal third carpal bone in the midcarpal joint ($p<0.05$). However, in the fetlock there was no significant difference between the thickness of the articular cartilage of the medial and lateral condyles of the distal cannon bone.

Conclusions. Articular cartilage thickness varied between joints and may be influenced by the size and function of the joint. Further studies are needed to investigate these relationships.

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Purine Nucleoside Phosphorylase Enhancement of Cancer Chemotherapy

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Purpose: Melanoma and mammary gland tumors are two of the most common types of malignant tumors found in dogs. The purpose of this study is to compare the effects of purine nucleoside phosphorylase (PNP) gene and drug 6-methylpurine-2'-deoxyribosid (MeP-dR) in causing tumor cell death verses MeP-dR alone in the treatment of canine melanoma and mammary gland tumors

Methods: Three canine melanoma (CML), 2 canine mammary tumor (CMT) cell lines and a canine histiocyte/macrophage (DH82) cell line were used for all experiments. The cell lines were transfected with Ad5 vectors that incorporated a luciferase reporter gene and infection was determined by luciferase expression. The cells were then infected with Ad5- PNP vector and assayed to determine PNP expressions. All cell lines were also incubated with MeP-dR for 2 different time periods and assayed to determine cell proliferation.

Results: All cell lines demonstrated transduction with an Ad5 vector. Cells were infected with Ad5-PNP and a PNP enzyme assay determined that all 6 cell lines showed PNP expression after infection. The cell lines were treated with 4 different concentrations of MeP-dR alone or infected with Ad5-PNP and treated with 4 concentrations of MeP-dR. A cell proliferation assay demonstrated that the combination of MeP-dR with Ad5-PNP was more effective at reducing proliferation than MeP-dR alone. The cells were also infected with Ad5-PNP alone and a PNP assay was done to determine PNP expression.

Conclusions: Adenoviral vectors are capable of transducing both canine melanoma and canine mammary tumor cells and can be used to develop cancer gene therapy applications for these tumors. Ad5-PNP is capable of infecting the cells with the PNP gene and will result in the expression of PNP. The combination of MeP-dR with an Ad5-PNP vector is more effective at decreasing viability of tumor cells than MeP-dR alone.

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Post-graduate / Faculty Platform Presentations

Targeted Cancer Nanomedicines

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Introduction. Cancer has become a national threat killing over 571,950 people annually according to the American Cancer Society's 2011 Cancer Statistics report. Although there has been significant progress in the treatment of cancer over the past 20 years, the overall five-year survival rate is still only 66%. Traditionally, surgery, radiotherapy and chemotherapy are the main treatment of cancer in routine practice. Conventional chemotherapy, which distributes free drug indiscriminately, results in severe systemic toxicity. Encapsulation of free drug in liposomes, micelles and other nanoparticles increases the accumulation of drug near the tumor site due to the enhanced permeability retention (EPR) effect of leaky tumor blood vessels and minimizes the toxic side effect of the drug. Targeting by ligands capable of specifically binding and internalizing tumor cells, can increase the efficiency and therapeutic effect of nanomedicines.

Methods. We have integrated the phage-display technology with a nanocarrier-based drug delivery and developed three phage-based cancer specific targeted drug delivery systems: 1) cancer specific phage protein targeted liposomal doxorubicin; 2) cancer specific phage protein targeted liposomes encapsulating siRNA and; 3) cancer specific phage protein encapsulating siRNA. The tumor-specific phage can be affinity selected from multibillion clone libraries by their ability to interact very specifically with cancer cell surface receptors. The phage based liposomal delivery systems explore the unique propensity of phage proteins to incorporate spontaneously into lipid bilayers of liposomes to form particles mimicking the structure of phage proteins in bacterial membranes. Similarly, self-assembly of major coat protein with siRNA results in "phage-like particles "or "nanophages" that mimic phage.

Results. These three platforms showed enhanced binding, internalizing and delivery of doxorubicin or siRNA to the cancer cells in comparision to non-cancerous cells and resulted in increased cytotoxicity or gene silencing in comparison to non-targeted drug carriers.

Conclusions. Further use of these approach may significantly enhance the screening of specifically targeted nanomedicines, result in dramatic acceleration of the translational process and change the field of cancer research through the development of more specific, safe and efficient targeted nanotherapeutics.

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Emergence of peripheral disease after brain-directed gene therapy in a feline model of Sandhoff disease.

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Introduction. Feline GM2 gangliosidosis is an animal model of Sandhoff disease (SD) that is currently untreatable in humans and fatal by 5 years of age. It is caused by a mutation within the β subunit of hexosaminidase (Hex), a lysosomal enzyme that cleaves neutral and charged throughout the brain and body. Intracranial adeno-associated viral (AAV) gene replacement of hexosaminidase α and β subunits results in a >four-fold increase in lifespan of Sandhoff cats, with marked attenuation of neurologic signs. This dramatic increase in life span due to successful treatment of the brain permits otherwise subclinical peripheral disease to emerge. Here we describe peripheral manifestations of feline Sandhoff disease after intracranial AAV gene therapy.

Methods. Monocistronic AAVrh8 vectors expressing feline Hex α and β subunits (1:1 ratio; 4.4×10^{12} g.c. total) were injected bilaterally into the thalamus and deep cerebellar nuclei of Sandhoff cats. Digital radiographs and ultrasounds were performed at the time of disease onset. Echocardiograms and abdominal ultrasounds were performed using a Phillips HD 11XE ultrasound (using a C85 or S124 probe). Cats were euthanized at humane endpoint and histopathology was performed in the heart, liver, kidney, small intestine and pancreas.

Results. Skeletal malformations include hemivertebrae, vertebral gibbus and spinal cord compression, pectus, elbow dysplasia, hip dysplasia, and patella luxation. Urinary glycosylaminoglycans (GAG) concentrations were 2433.8±946.9mg GAG/mg Creatine (Cr) for untreated SD cats, 654±254 mg GAG/mg Cr for AAV- treated SD cats and 132.4±48.8mg GAG/mg Cr for normal cats. Gastrointestinal disturbances include proximal GI ileus with a lymphocytic plasmacytic phenotype and chronic recurrent pancreatitis. Vacuolation of pancreatic acinar cells is also apparent. Cardiac abnormalities include truncation and distortion of valves with both mitral and AV valve dysplasia and right atrial enlargement. Histopathologic changes include occasional cardiomyocyte and purkinje cell vacuolation, myoxmamtous foamy appearance of valvular and stromal cells, and vacuolation of vascular smooth muscle cells. Focal areas of infarction and myonecrosis have been noted. Cellular vacuolation was noted within hepatocytes and splenic sinusoidal cells.

Conclusions. Skeletal abnormalities, gastrointestinal and cardiovascular dysfunction are a significant source of morbidity in AAV-treated SD cats. Therefore characterization of peripheral disease in the feline model is important to determine areas to be targeted in future therapeutic strategies and as to inform potential outcomes in future human clinical trials.

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Echocardiographic findings in kittens and cats with GM1 and GM2 Gangliosidosis

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Introduction. Metabolic cardiomyopathies have been well documented in the literature. Cardiac changes reported with GM1 gangliosidosis include congestive cardiomyopathy with a decrease in contractility, thickening of the mitral, tricuspid, and to a lesser degree aortic valve. In patients with GM2 gangliosidosis, Sandhoff's disease, left ventricular hypertrophy with dilatation, malformed chordae tendineae, and thickened valves due to fibrous tissue has been documented. Over the past several years, several of the kittens and cats with GM1 and GM2 gangliosidosis have presented for echocardiogram due to signs of cardiac disease. Echocardiograms were performed in colony animals if cardiac enlargement or sign of heart failure was noted on thoracic radiographs or following detection of a heart murmur. The echocardiographic findings in colony animals were compared to the published literature.

Methods. A Phillips iE33 ultrasound machine with a S12-4 MHz phased sector array probe was used after placing warmed ultrasound gel on the thoracic wall. Thirteen kittens or cats were scanned either in sternal or lateral recumbency with minimal restraint and no sedation. Echocardiograms were performed on 5 patients with GM1 and 8 patients with GM2 gangliosidosis.

Results. The 2/3 GM1 kittens that progressed to heart failure showed severe decrease in contractility, along with pericardial, pleural, and peritoneal effusion on the echocardiogram. The other GM1 kitten that progressed to heart failure had a normal contractility but a greatly enlarged left atrium and a small volume of pericardial effusion. In the 2 remaining GM1 cats, the contractility was normal; the right atrium appeared larger than the left atrium with variable degrees of turbulence noted within this atrium. In the 8 cats with GM2 gangliosidosis, the contractility was normal, 6/8 had an enlarged aorta or aortic arch and increase size of the right atrium with variable degrees of turbulence noted within this atrium.

Conclusions. The GM1 echoes showed some cardiac changes similar to the reported literature. The GM2 echoes in 6/8 patients showed enlarged aorta or aortic arch which has not been previously reported with this metabolic disorder. The cardiovascular changes noted in the GM2 cats are similar to published finding reported in feline patients with mucopolysaccharidosis I and VI and may be explained by deficiency of the GM2 enzyme, which normally cleaves multiple substrates in peripheral tissues. Right atrial changes noted in both GM1 and GM2 cats need to be further investigated.

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Canine PBMC NSG mice: A *in vivo* model of the canine immune system

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Introduction. Murine models capable of engraftment of xenogenic tissues represent a major advancement in our ability to generate physiologic models of human and animal diseases. One such model system involves use of NODscid IL2Rγ -/- (NSG mice) that has been utilized extensively in generation of models of HIV infection, cancer, drug toxicities, transplantation studies, among many others. The mutation in the IL2γ receptor renders signaling through all IL2 family receptors non-functional resulting in suppression of the innate immune system including a complete deficiency in NK cells, which allows for better engraftment. **Our aim was to validate a model engrafted with canine (PBMCs) from healthy donors to determine whether stable engraftment with canine PBMC's is possible.**

Methods. PBMC's from Labrador Retrievers were purified from whole blood by Ficoll density centrifugation and 10^7 were injected by the intraperitoneal route into NSG mice 8 hours after irradiation with a 100cGy dose of γ radiation. On 1, 9, 16, 28, 36 42 and 52 days post engraftment the total canine WBC inoculum and its subpopulations were determined by flow cytometry using antibodies specific to helper T cells (CD45+ CD4+), cytotoxic lymphocytes (CD45+ CD8+), regulatory T cells (CD45+ CD4+ Foxp-3+), and B cells (CD45+ IgG+).Onset of xenogenic graft versus host disease (GVHD)was assessed by measurements of weight where a 15% reduction in body weight or the presence of marked anemia, and thrombocytopenia all indicative of xenogenic GVHD were used as criteria for humane euthanasia.

Results. At 1 day post engraftment canine CD45+ lymphocytes were detectable within the peritoneal cavity. Beginning at 9 days, canine CD45+ lymphocytes were detectable in the blood, bone marrow, and spleen with numbers of canine CD45+ lymphocytes in the spleen and bone marrow peaking at 28 days. Characterization of canine CD45+ lymphocyte subset diversity revealed an early predominance of helper and regulatory T cells in contrast to increasing proportions of CTL's and IgG+ B cells that peaked at 28 days. Canine specific IgG was detectable beginning at 9 days post engraftment and increased in concentration during the engraftment period. Clinical symptoms associated with xGVHD were consistently noted around 42 days post engraftment. In the most severely affected mice there was evidence marked anemia and regeneration, agglutination and spherocytes, despite no obvious pathology upon surveys of mouse tissues including the bone marrow.

Conclusions. NSG mice successfully engraft with canine lymphocytes from healthy donors. The inverse relationship between decreasing proportions of helper and regulatory T cells in comparison CTLs and antibody secretion may suggest a potential role in the development of humoral and cell-mediated immunity to mouse auto antigens. At 42 days post engraftment engrafted mice displayed severe anemia, thrombocytopenia, and in ¼ of cases immune mediated hemolytic anemia. These findings suggest that NSG mice have the potential to be a model with which we can examine canine immune responses; however its impact may be blunted by the onset of xGVHD.

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Veterinary Student Poster Presentations

Stearidonic acid, an omega-3 fatty acid, inhibits human prostate cancer cell viability

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Introduction: Prostate cancer (PCa) is a leading cause of cancer deaths among men in the United States. The marine-based omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid [EPA; 20:5 (n-3)] and docosahexaenoic acid [DHA; 22:6 (n-3)], have antitumorigenic properties but are inefficiently converted from their primary metabolic precursor, alpha-linolenic acid (ALA; 18:3 (n-3)]. Stearidonic acid [SDA; 18:4 (n-3)], a plant derived omega-3 abundant in echium and borage oils, is an EPA and DHA precursor that humans can metabolize making it an alternative source to marine-based PUFAs. The purpose of this study was to determine the antiproliferative and anti-androgenic properties of SDA in human prostate cancer cell lines.

Methods: Cell viability/proliferation was determined with MTT cell growth assays. Nuclear Factor Kappa B (NF-kB) inhibition was achieved with a luciferase reporter assay using TNF-alpha as a positive control. Androgen receptor (AR) expression was performed using immunocytochemistry.

Results: We treated cultures of three human prostatic cell lines with SDA to determine its effect on cell viability, AR expression, and NF-kB activity. We found that SDA at 50 micromole/L and higher concentrations killed androgen-dependent LNCaP and androgen-independent PC3 cancer cell lines but not RWPE1 normal prostate epithelial cells. The cell death was significantly higher in PC3 compared with LNCaP cells. Immunocytochemistry showed that SDA treated LNCaP cells down regulated testosterone-induced AR expression. Likewise, SDA treatment decreased TNF-alpha-induced NF-kB activity in LNCaP cells.

Conclusions: These results suggest that SDA could be used as a cytotoxic dietary-supplement for PCa treatment particularly in the difficult to treat androgen-independent PCa.

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Evaluation of the shape and depth of the collateral groove of the foot as a method to predict the position of the distal phalanx within the hoof capsule

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Purpose: To determine the accuracy of collateral groove depth measurements in predicting the thickness of the sole and the orientation of the distal phalanx within the hoof capsule.

Introduction: The foot is the most common site of pain causing lameness in horses. Physical examination for purchase or for lameness often begins by visually inspecting the foot. Based on dissection studies of the foot there appears to be a constant relationship between the external characteristics of the collateral grooves of the hoof capsule and internal hoof structure. The collateral grooves apparently run parallel to and a fixed distance from the solar surface of the distal phalanx in the dorsal half of the foot and the same distance from the base of the collateral cartilages in the palmar half of the foot.

In the healthy foot with adequate depth of sole, the collateral groove at the apex of the frog has been described to be 10 – 20 mm from the ground plane (Ramey, 2011). Clinical observation has shown that the orientation of the collateral groove in the front half of the hoof parallels the palmar/plantar angle of the distal phalanx (Schumacher et. al., 2012). We hypothesized that anatomical features of the hoof that can be predicted based on characteristics of the collateral grooves are: depth of sole, distance of the distal phalanx from the bearing surface, and the palmar/plantar angle.

Procedure: Using two commercial tools, five points were measured along the collateral groove on each hoof of 24 horses (n=96 hooves). Lateral radiographs were taken on all four limbs of each subject. Statistical analyses were made comparing the external measurements and the radiographic measurements.

Results/Conclusion: Depth of the collateral groove at the apex of the frog was highly associated with subsequent radiographic measurements of sole depth ($p<0.0001$) and P3 suspension ($p<0.0001$). Depth of the collateral groove at the apex of the frog was highly associated with subsequent radiographic measurements of the palmar angle ($p<0.0001$).

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Role of Adiponectin in Niacin-Mediated Reduction in Hepatic Triglyceride Storage

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Introduction. Along with the rising trends in excessive calorie consumption, there has been an increased incidence of non-alcoholic fatty liver disease in recent years. Research from our lab shows that although mice fed a HFD diet have increased lipid triglyceride accumulation in the liver, mice that are fed a HFD and are concurrently treated with niacin are protected from this lipid accumulation. In addition, previous research has shown that niacin administration causes increased levels of serum adiponectin and that hepatocytes possess receptors for this protective adipokine. It is also known that adiponectin binding to its receptors causes an increase in pAMPK and PPARα, which play a role in lipid metabolism. In this study, we hypothesized that niacin's effects on hepatic triglyceride accumulation are mediated by adiponectin binding to its receptors in the liver and subsequent changes in protein and gene expression. Specifically, we hypothesized that gene expression of SREBP1c, FAS, DGAT2, and ACC1, enzymes and transcription factors involved in lipid synthesis, will be increased with HFD but decreased with concurrent niacin treatment. Furthermore, we expect gene expression of SCD1 and PPARα and protein expression of pAMPK to be decreased in HFD but increased with niacin treatment, as they play a role in fatty acid metabolism.

Methods.

Subjects. Thirty-two male mice (Charles River Laboratories) were used in this study. Mice were fed either normal chow or HFD for the duration of the study. After 6 weeks of their respective diets, half of the mice in each group were given vehicle (water) while half were given niacin treatment (200 mg/kg/day in water). After 5 weeks of vehicle/niacin treatment, mice were euthanized and tissues were collected.

Gene and Protein Expression. Gene expression was evaluated using RT-PCR. Protein expression was evaluated using Western Blot analysis with fluorescently labeled antibodies.

Statistical Analysis. A One-Way ANOVA was performed using a significance level of $p<0.05$. When differences were observed, a Bonferroni post-hoc test was performed.

Results. Gene expression of ACC1, FAS, SREBP1c, DGAT2, PPARα, SCD1, AdipoR1 and AdipoR2 was not affected by niacin administration. However, AMPK activation was significantly increased with niacin treatment.

Conclusions. Gene expression of major regulatory enzymes or transcription factors involved with lipid synthesis was not significantly altered. However, pAMPK protein expression was significantly increased in both HFD and niacin treatment, possibly indicating signaling through the adiponectin receptor. Since other factors can influence AMPK activation, the role of adiponectin in niacin's TG abrogation needs to be further investigated.

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Pregnenolone 16-alpha Carbonitrile Impairs Testosterone Biosynthesis in Rat Leydig Cells

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Introduction: Testicular Leydig cells (LC) are the major source of the male sex hormone testosterone (T), which supports the male phenotype. Several therapeutic drugs and environmental chemicals are known to disrupt T homeostasis. Importantly, some of these drugs and chemicals can activate pregnane xenobiotic receptor (PXR), which is a ligand-dependent orphan nuclear receptor that regulates the expression of the enzymes/proteins involved in the metabolism of endobiotics and xenobiotics. Notably, PXR activation has been shown to disrupt corticosteroid hormone homeostasis. It is therefore possible that PXR activation in LC may lead to impaired T homeostasis. In this preliminary study, we sought to determine whether pregnenolone 16-alpha carbonitrile (PCN), an agonist of rodent PXR, affects T biosynthesis in LC.

Methods: Rat LC (isolated from 35 day-old male Long Evans rats) as well as MA-10 mouse Leydig tumor cells were treated with either dimethyl sulfoxide (0.1%) or PCN (10 μM) for 24h. The cells were then either unstimulated (basal) or stimulated with luteinizing hormone (LH; 100ng/ml) for additional 3 h before performing radioimmunoassays to measure the concentration of secreted T or cAMP (intracellular and extracellular). Western blots were conducted to determine the protein expression.

Results: Treatment of LC with PCN decreased both basal and LH-stimulated cAMP levels and T secretion. Additionally, PCN treatment resulted in the downregulation of steroid acute regulatory protein (StAR), cytochrome P450 17A1 (CYP17A1), 3β-hydroxysteroid dehydrogenase (3β-HSD), and 17β-HSD, suggesting that PCN impairs T biosynthesis in LC by downregulating the expression of the enzymes/proteins involved in T biosynthesis. PCN, however, did not alter the expression of CYP19, which converts T to estrogen (E), suggesting that PCN-decreased T production in LC may not be due to increased conversion of T to E.

Conclusions: These preliminary observations are consistent with the conclusion that PCN impairs T biosynthesis in LC by decreasing cAMP levels and by downregulating the expression of the enzymes/proteins involved in T biosynthesis. Future studies will be directed to demonstrate whether PCN impairs T biosynthesis via PXR in LC.

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Analysis of two formulations of ceftiofur (Naxcel® and Excede®) in the seminal plasma of normal stallions

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Introduction. Treatment of infections within the reproductive tract of stallions has many challenges. One such challenge is effective treatment with necessary antibiotics. Little is known as to which antibiotics can be administered systemically and successfully enter the reproductive tract, or the dosage needed to achieve effective concentrations to treat these infections.

Methods. In this experiment six healthy stallions were given two formulations of ceftiofur, either Naxcel®, a daily injectable antibiotic, or Excede®, a long acting, slow release formula, in a cross over design, intramuscularly at the manufacturer's recommended dose for ten days (Naxcel 2.2mg/kg daily, Excede 6.6 mg/kg twice 96 hours apart). Semen and plasma were collected from all stallions every other day to determine the concentration of ceftiofur present in seminal plasma, and correlate it to plasma concentrations.

Results. Ceftiofur was identified in the seminal plasma of all stallions given Naxcel® and Excede®. The seminal plasma concentration of ceftiofur in stallions administered Naxcel® tended to be higher than those receiving Excede®. The average ceftiofur concentration within the seminal plasma for all stallions was 177.0 ng/mL for those receiving Naxcel®, and 87.9 ng/mL for those receiving Excede®. Most samples were below the target minimum inhibitory concentration (MIC) for plasma (200 ng/mL) with only 18% of all samples reaching 200 ng/mL or above.

Conclusions. Both ceftiofur formulations administered at the recommended dose were able to cross into the stallion's reproductive tract, but failed to consistently reach the predetermined ceftiofur target MIC (200 ng/ml) within the seminal plasma. Further studies with a higher dosage of ceftiofur are necessary to determine if the target MIC could be reached within seminal plasma with higher blood plasma concentrations.

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Stearidonic Acid Sensitizes Human and Canine B-Cell Lymphoma Cells to Vincristine and Cyclophosphamide

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Introduction: Malignant lymphomas, including B-cell lymphoma, account for one of the leading causes of cancer deaths in humans and canines. Both human and canine B-cell lymphomas have similar tumor biologies and most often the same therapies are used for treatment. Although chemotherapy is the most effective therapy for treating human and canine B-cell lymphomas, routinely used chemotherapeutics, such as vincristine (VCR) and cyclophosphamide (CPA), lose their antitumor efficacy during the later stages of treatment due to chemoresistance, resulting in recurrence of the lymphoma. Therefore, there is an immense need to discover novel therapeutic approaches to combat against chemoresistance. Some marine-based polyunsaturated fatty acids (PUFAs) have long been shown to exhibit antitumorigenic properties in a variety of human and canine cancers. Recently, a plant-based stearidonic acid (SDA), which serves as an alternative source to marine-based PUFAs, has been shown to exert antitumorigenic effects. The purpose of the current study was to determine the antiproliferative and chemosensitizing properties of SDA in human and canine B-cell lymphoma cells.

Methods: Ramos (human B-cell lymphoma cells), 17-71 and GL-1 (canine B-cell lymphoma cells), normal canine fibroblasts (NCF), and canine mammary tumor (CMT12, CMT27 and CMT28) cells were used in the current study. Cell viability was measured using ATP-based CellTiter-Glo luminescent cell viability assays after treating the cells for 24 h with dimethyl sulfoxide (0.1%), ethanol (0.1%), SDA (100-500 µM), vincristine (VCR; 10 µM), cyclophosphamide (CPA; 10 µM) or doxorubicin (DOXO; 10 µM). To determine the synergistic antitumor effect of SDA and chemotherapeutics, the cells were treated with SDA and VCR or CPA for 24 h before measuring the cell viability.

Results: SDA significantly reduced the viability of both the human and canine B-cell lymphoma cells in a concentration-dependent manner. In contrast, SDA did not affect the viability of normal canine fibroblasts (NCF) or canine mammary tumor cells, suggesting that SDA selectively inhibits the growth of the B-cell lymphoma cells. The B-cell lymphoma cells were found to be highly sensitive to DOXO yet moderately or fully resistant to VCR or CPA. Interestingly, VCR or CPA in combination with SDA markedly reduced the viability of the B-cell lymphoma cells, suggesting that SDA sensitizes the human and canine B-cell lymphoma cells towards chemotherapy.

Conclusions: The results are consistent with the conclusion that SDA inhibits the growth and enhances the chemosensitivity of both human and canine B-cell lymphoma cells to VCR and CPA. The results from this study suggest that SDA could be used as a dietary supplement to prevent the recurrence of human and canine B-cell lymphoma by enhancing the antitumor efficacy of VCR or CPA.

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Testing a Synthetic Antioxidant on a Mitochondrial Toxin in NIH/3T3 Mouse Fibroblast Cells

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Introduction. Recent research has focused on lessening the effects of aging by targeting mitochondria and their role in apoptosis. The mitochondrial toxin, rotenone, was previously used *in vivo* as model of aging and in the evaluation of a possible therapeutic antioxidant, PMX-500F. The purpose of this study was to further determine the effects of rotenone, a common pesticide and complex I inhibitor, and the antagonism of PMX-500F on NIH/3T3 cells. Specifically, the primary goals were to determine the mechanism of action of PMX-500F and to define a set of *in vitro* parameters for future studies on the antioxidant.

Methods. Cells were treated with 2 µM rotenone and 2 µM PMX-500F for 24 and 48 hours. Cells were evaluated with photomicrographs, Hoechst 33342 stain, reactive oxygen species (ROS) measurement with dichlorofluorescein diacetate (DCF-DA) fluorescence, and western blotting.

Results. After 24 hour treatment, a 21.1% increase in ROS values were seen in cells with rotenone, 9.9% in cells with PMX-500F, and 15.2% in cells with both rotenone and PMX-500F. After 48 hour treatment, ROS values increased 40.7% with rotenone, 23.4% with PMX-500F, and 63.5% with both. Apoptosis in 24 hour treatments indicated an increase of 23% with rotenone, 38% with both, and a decrease of 0.5% with PMX-500F. After 48 hours, apoptosis was 36% with rotenone, 28% with both, and -3% with PMX-500F. Values represent a comparison of untreated cells. Western blotting showed the highest increase in cleaved caspase-3 in both rotenone and rotenone plus PMX-500F cells for 24 hour treatment. 48 hour treatment showed the highest increase in cleaved caspase-3 in rotenone plus PMX-500F cells.

Conclusions. The results illustrate that PMX-500F failed in rescuing NIH/3T3 cells from the effects of rotenone at the chosen concentration and time points. An incidental finding was that PMX-500F alone lowered ROS (as compared to rotenone), apoptotic factors, and cell overgrowth. Further studies need to be performed to definitively characterize antioxidant effects on mitochondrial function.

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Correlation between examination and Mimics 3-dimensional reconstruction of the equine heel

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Introduction. Traditionally, equine foot lameness has been attributed to pathology of the bones, synovial structures, tendons and ligaments, and the lamina of the foot. Recently it has been proposed that healthy heel soft tissue structures, especially the digital cushion, play a primary role in equine soundness. The goal of this study was to determine if physical examination, radiographic, and ultrasonographic measurements are predictive of measurements determined using Mimics®[a] three-dimensional reconstruction of the equine heel.

Methods. Eight left front feet were collected from Thoroughbred horse cadavers. The feet were imaged using computed tomography (CT) and magnetic resonance imaging (MRI). Using Mimics®, three-dimensional images were constructed and volumes were determined of the following anatomical structures: collateral cartilages, digital cushion, middle and distal phalanges. A physical examination scoring system for the equine heel was established. Physical examinations and diagnostic imaging were performed on the same feet. The relationships between clinical exam measurements and Mimics® values were explored using simple linear regression.

Results. Using the parameters designed for this study, each foot was ranked based on predicted volume. The volumes of the digital cushion and distal phalanx were determined by three-dimensional reconstruction and compared as a ratio. The feet were then ranked based on the ratios of digital cushion to distal phalanx volumes found using medical image processing software. Feet were also ranked using a subjective evaluation of the lateral radiographs based on estimated heel area. It was found that all three rankings correlated, but the physical exam heel score rankings were most similar to the Mimics® rankings. We also noted that a number of individual ultrasound and physical examination measurements were found to significantly predict the volume of the digital cushion.

Conclusions. Although physical and radiographic examination did not perfectly predict Mimics® findings, they did categorize these horses into three distinct categories: low, medium, and high volume. Additional physical and clinical examination parameters are needed to accurately evaluate the maturity of the collateral cartilages and the amount of fibrocartilage in the digital cushion. Data from this study serves as a very preliminary screening for clinical examination measures that may serve as predictor variables for anatomical characteristics of the equine heel.

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Elevated prolactin levels increase neurogenesis in the male rat

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Introduction. Neurogenesis commonly occurs in the Subventricular zone (SVZ) adjacent to the lateral ventricle and also in the dentate gyri of the hippocampus in the adult brain. The newly generated SVZ neuronal precursor cells migrate in chains along the rostral migratory stream to the subependymal zone of the olfactory bulb, and then radially through the olfactory bulb where they differentiate into inhibitory interneurons. The new neurons populate and function predominantly as gamma-aminobutyric acid (GABA) interneurons in the overlying granule cell layer, affecting odor processing. Prolactin has been shown to increase SVZ neurogenesis in dams. It is thought to be associated in offspring recognition and subsequent increased maternal behavior. The purpose of this study is to determine if prolactin induces neurogenesis in male rats.

Methods. Ten adult male Sprague Dawley rats were subcutaneously implanted with omostic alzet pumps. Five rats received prolactin (625 mcg/hr) for seven days while the remaining five received saline vehicle. Animals also received daily injections of 5-ethynyl-2'-deoxyuridine (EdU; 10mg/kg, i.p.). EdU is a fluorescent-tagged nucleoside analog of thymidine which is incorporated into dividing cells. Animals were perfused and brains were sectioned using a cryostat. Labeled cells were visualized for EdU positive cells. Tissue was viewed using an epifluorescent and confocal microscope. If present, EdU positive neurons were classified based on neurotransmitter markers, using Glutamic acid Decarboxylase (GAD67) and Vessicular Glutamate Transporter (VGLUT) antibodies.

Results. Prolactin treated animals had a higher average number of EdU positive cells in both the olfactory bulb and the dentate gyrus of the hippocampus when compared to the vehicle treated animals ($p \leq 0.05$). A significant difference could not be detected between the two groups in the SVZ.

Conclusions. This work is the first evidence of prolactin- induced neurogenesis in male rats. We have demonstrated that prolactin treated male rats had higher rates of neurogenesis in both the olfactory bulb and dentate gyrus compared to the vehicle treated animals. Further work will focus on continued phenotyping of these new neurons as either GABAnergic or glutamatergic. Co-localization of EdU and GAD67/VGLUT is necessary for a positive diagnosis.

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A Regression Analysis Study for Prediction from Colic in the Horse

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Introduction. Colic in horses is the common term for abdominal pain caused by gastrointestinal disorders. It is a leading cause for examination by a veterinarian and is also the most common cause of death in all horses. The cause of colic is highly variable and can range from a minor sympathetic spasm to a severe strangulation lesion. The prevalence of this disorder lead us to investigate a predictive model for death in horses with colic. This study intended to focus on exam findings based on equipment and diagnostic techniques readily available in the field. The goal of this study was to provide ambulatory practitioners with a tool to aid in the decision for referral and treatment of horses with acute abdomen.

The hypothesis was that a statistical model could be used to determine a predictive model for death from colic.

Methods. Data was collected from medical records for all horses admitted for "colic" from January 2006 to June 2012. A total of 256 horses were identified. 121 cases were excluded for various reasons to allow for statistical comparison of equal groups in the study.

Variables analyzed include: age, sex, heart rate (HR), respiratory rate (RR), temperature, altered gastrointestinal (GI) sounds, mucous membrane (MM) color, pain on arrival, transrectal palpation of distension of the large or small intestine, net gastric reflux, and survival. HR was further divided into low and high HR (<53 or >54 beats per minute {bpm}) and low and high RR (<24 and >25 breaths per minute {brpm}). All variables were assessed upon arrival of the patient to the hospital.

Statistical analysis using Student's T-tests was performed to determine significant differences ($P \leq 0.05$) when looking at survivors versus non-survivors in the population studied. Linear regression analysis was used to further assess variables individually and allow for multiple comparisons.

Results. Mortality was higher in geriatric horses ($P=0.002$) and heavier horses ($P=0.021$), and also in horses with a high HR ($P<0.001$), high RR ($P=0.035$), decreased GI motility ($P=0.002$), abnormal MM color ($P<0.001$), and evidence of abdominal pain ($P<0.001$). Transrectal palpation of small intestinal distention was also a negative prognostic indicator ($P<0.001$), as was the presence of >4 liters of net gastric reflux ($P=0.026$).

Linear Regression Analysis indicated the variables able to significantly predict survivorship included: age ($P=0.02$), HR ($P=0.009$) and high HR ($P=0.0275$). Modeling for interaction noted that no combinations of these variables were better able to predict survival than the variables alone ($P<0.05$). However, there was a trend for age and HR to significantly interact ($P=0.07$). This data suggests that for this population, only HR may be the best predictive variable for survival from colic.

Conclusions. Several variables identified differ statistically between horses that died and survived. Based on this study, a HR>54 bpm may allow ambulatory veterinarians to provide a more accurate prognosis for survival from a colic episode.

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Use of an Adenoviral Vector to Deliver PNP to Canine Melanoma and Mammary Gland Tumor Cells

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Purpose: Melanoma and mammary gland tumors are two of the most common types of malignant tumors found in dogs. The purpose of this study is to determine if an adenoviral vector (Ad5) can be used to deliver the purine nucleoside phosphorylase (PNP) gene to canine melanoma and mammary tumor cells and to show that the PNP gene in combination with the drug 6-methylpurine-2'-deoxyribosid (MeP-dR) will cause tumor cell death.

Methods: Three canine melanoma (CML), 2 canine mammary tumor (CMT) cell lines and a canine histiocyte/macrophage (DH82) cell line were used for all experiments. The cell lines were transfected with Ad5 vectors that incorporated a luciferase reporter gene and infection was determined by luciferase expression. The cell lines were then tested for the presence of mycoplasmas using a Takara PCR Mycoplasma Detection Set and treated with Plasmocin. The cells were then infected with Ad5- PNP vector, incubated with MeP-dR and assayed to determine both PNP expression and cell proliferation.

Results: All cell lines demonstrated transduction with an Ad5 vector. All 6 cell lines initially tested positive for mycoplasma contamination and were treated with Plasmocin. After 17 days of treatment, all 6 cell lines tested negative for mycoplasma. Cells were infected with Ad5-PNP and a PNP enzyme assay determined that all 6 cell lines showed PNP expression after infection. The cell lines were treated with 4 different concentrations of MeP-dR alone or infected with Ad5-PNP and treated with 4 concentrations of MeP-dR. A cell proliferation assay demonstrated that the combination of MeP-dR with Ad5-PNP was more effective at reducing proliferation than MeP-dR alone.

Conclusions: Mycoplasma contamination is a serious problem in cell culture, but PCR can identify contaminated cells lines and Plasmocin is effective at treating the contamination. This is critical as mycoplasma expression of PNP can create false positive results in the assays. Adenoviral vectors are capable of transducing both canine melanoma and canine mammary tumor cells and can be used to develop cancer gene therapy applications for these tumors. The combination of MeP-dR with an Ad5-PNP vector is more effective at decreasing viability of tumor cells than MeP-dR alone.

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Examination of Biomarkers of Neurodegeneration in Feline GM2 Gangliosidosis

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Introduction. GM2 gangliosidosis (GM2) is a lipid storage disease in which deficiency of a hydrolytic enzyme leads to accumulation of GM2 ganglioside in lysosomes, resulting in neurodegeneration and subsequent inflammatory responses. GM2 cats provide a model to study the effectiveness of gene therapy to deliver the insufficient enzyme and impede disease progression. Biomarkers of disease progression are desired to characterize disease advancement and measure success of therapy. It is known that pro-inflammatory cytokines, such as TNF-alpha, and chemokines, such as MIP-1alpha, are readily produced in chronic inflammatory states associated with neurodegeneration. Therefore, TNF-alpha, MHCII, and MIP-1alpha were analyzed as potential biomarkers in feline GM2 gangliosidosis.

Methods. Cats with GM2 were treated by intracranial injections of an AAV vector encoding feline hexosaminidase. Samples were collected from 3 cats at 16 weeks post-treatment for short-term analysis while remaining cats were followed to humane end point (9.8-21.5 mos.).

Results. There was no significant difference in gene expression measured by qRT-PCR or protein levels detected by ELISA of TNF-alpha between normal and GM2 affected cats. Immunohistochemistry (IHC) demonstrated an increased intensity of TNF-alpha in the neurons of GM2 affected cats. Gene expression levels of MHCII were elevated 1.8-9 fold over normal in GM2 affected cats. AAV-treated GM2 cats had levels ranging from 0.6-5.3 fold over normal controls. IHC for MHCII appeared to correlate to gene expression. MIP-1alpha gene expression levels were 2.4-4.6 fold over normal in untreated GM2 cats and appeared to increase in short-term AAV-treated cats to an average of 7.4 fold over normal. In long-term AAV-treated cats, MIP-1alpha levels returned to normal.

Conclusions. Homogeneity of TNF-alpha levels and substantial variability of MHCII levels exclude them as potential biomarkers, but MIP-1alpha remains a potential candidate.

Graduate Student Poster Presentations

Role of Microglia Cells in the Pathogenesis of Feline GM2 Gangliosidosis

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Introduction. Microglia cells are the primary resident immune cells of the CNS. Non-activated, resting microglia persist with a ramified morphology, consisting of a small, round cell body with long, branching processes used to survey for bacteria, viruses, cell debris, or degenerating neurons. Once activated, microglia assume an ameboid morphology capable of phagocytosis, express MHCII to present antigens and activate T-cells, and secrete pro-inflammatory molecules. A well established feline model of GM2 gangliosidosis (GM2) provides a means to further define the role of microglia in neurodegeneration. Additionally, therapeutic intervention with an AAV vector encoding the deficient enzyme, Hexosaminidase A, (AAV-Hex), has proven to significantly delay disease onset, and thus should curtail the activation of microglia.
Methods. Four-week old cats affected with GM2 gangliosidosis were treated by bilateral thalamic and deep cerebellar nuclei (DCN) injections of AAV-Hex. Three cats were euthanized at 16 weeks post-treatment for short term analysis while three remaining cats were followed to humane endpoint. For immunohistochemistry, 6µm paraffin-embedded sections were blocked for 1hr with 5% normal horse serum, treated for 1hr with mouse anti-feline MHCII (1:4) or rabbit anti-human/rat/mouse Iba1 (1:100) followed by biotin-labeled horse anti-rabbit/mouse IgG for 1hr, ABC Reagent for 30 min, and visualized with DAB substrate.
Results. Untreated GM2 cats exhibited an overwhelming population of activated microglia characterized by ameboid morphology and expression of MHCII and Iba1 (a marker of both resting and activated microglia). In comparison, a normal, age-matched control displayed non-activated microglia of the ramified nature and lacked positive staining for MHCII. These results were consistent throughout the cerebrum, cerebellum, and spinal cord. Three GM2 cats treated with thalamic and DCN injections of AAV-Hex and euthanized 16 weeks post-treatment, with negligible indication of disease, displayed histological features of microglia indistinguishable from normal cats. One cat euthanized at 10 months of age due to non-neurologic reasons had a population of microglia almost entirely of the resting, ramified nature consistent with successful delay of disease onset. However, two cats treated for long-term evaluation (>1yr), with pronounced symptoms of disease, revealed activated microglia typical of late disease stages. Additionally, in one cat treated in the thalamus but not the DCN, resting microglia predominated in the cerebrum, while activated microglia were readily apparent in the cerebellum.
Conclusions. Excess ganglioside in the neurons of GM2 cats leads to activation of microglia, characterized by ameboid morphology and expression of MHCII. However, AAV mediated gene delivery delays disease onset and activation of microglia. Chronically activated microglia present during late stages of disease likely contribute to inflammation, cytotoxicity, and accelerate disease progression.
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Fluroquinolone-induced mutations in SoxS, a regulator of AraC/XylS efflux pump activity

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Introduction. *Escherichia coli* is a major cause of urinary tract infections in dogs and cats, and fluoroquinolones are among the drugs most commonly used to treat UTI. Nonetheless, a substantive increase in fluoroquinolone resistance in companion animal E. coli isolates has been reported (Shaheen et al., 2010) including that associated with multi-drug resistance (MDR). A contributing mechanism by which E.coli generates antimicrobial resistance is overexpression of efflux pumps. In the act of protecting themselves against endogenous and exogenous toxins, mutations in these pumps allow further development of resistance to antimicrobials. AcrAB-TolC is the main efflux pump associated with fluoroquinolone resistance and its expression is regulated by members of the AraC/XylS family of transcriptional activators, such as SoxS which is regulated by the transcriptional activator SoxR (Martin et al., 2002). Fluoroquinolone therapy has shown to develop mutations in soxR resulting in the overexpression of soxS and thus overexpression of the efflux pump, but more has to be done regarding the effects of mutations in soxS on drug resistance. The goal of this study is to evaluate different generations of fluoroquinolones at different concentrations and for distinct exposure times depending on whether they lead to the development of mutations in soxS.

Methods. Broth macrodilution was performed in order to expose *wt E. coli* to increasing two-fold concentrations (0.06 µg/mL to 4 µg/mL) of Ciprofloxacin, Marbofloxacin, Pradofloxacin and Gatifloxacin. Samples were taken from each well and streaked onto trypticase agar after 30, 60, 90 and 120 minutes of antimicrobial exposure. Plates were then incubated for 24 hours at 35°C. A positive control with a known soxS mutation and a negative control were plated as well. A checkerboard method was used in order to compare different antimicrobial concentrations with their respective exposure times. PCR was performed on bacterial DNA in order to amplify soxS (324bp). The PCR amplicons were purified and sent off for sequencing at Macrogen,Inc. Both forward and reverse strands were sequenced.

Results. Multialign was used to compare *wt* E.coli soxS sequence pre-fluoroquinolone exposure (negative control) to each individual sample sequence. Marbofloxacin and Pradofloxacin exposure did not present any mutations within soxS. On the other hand, *wt* E.coli exposed to Gatifloxacin for 30 mins at 1µg/mL contained 5 single point bp mutations in the forward strand of soxS. Interestingly, Ciprofloxacin exposure for 120 mins at 4 µg/mL and 0.06 µg/mL contained 14 and 33 single point mutations respectively in the forward strand of soxS.

Conclusions. This study is interested in evaluating how different generations of fluoroquinolones may have a different impact on the contribution to drug resistance and also if the variables of time and concentration contribute to mutations in soxS. From the results obtained, we can determine that Ciprofloxacin, a second generation fluoroquinolone, presented mutations in a time dependent manner. In order to determine the impact of these mutations, mRNA levels for soxS and AcrAB need to be further analyzed.

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Novel genetic defects in p16/INK4A and differential expression profile of CKI tumor suppressors in canine melanoma and mammary tumor models

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Introduction. Cell cycle progression is tightly regulated by group of proteins called cyclin-dependent kinase inhibitors (CKIs) that also act as endogenous tumor suppressors in many mammalian tumors. Two families of CKIs –INK4 (p16, p15, p18, p19) and CIP-KIP (p21, p27, p57), block the G1/S phase transition of the cell cycle. p16/IKN4A is an important tumor suppressor gene defects in which have been associated with a number of cancers including human and canine breast cancers. p16 is encoded from the INK4A gene locus and then alternatively spliced into two distinct messages called p16 and p14ARF that share two common exons (2 and 3) but different first exons- 1α and 1β respectively. In dogs, the p16 transcript has not been fully elucidated since there are no full-length mRNAs or express sequence tags available that would completely define this transcript. An extremely GC-rich content has also made it difficult to clone and sequence and it is missing from the published canine genome. The complete sequence information of p16 is a critical step to assess gene expression and mapping of mutation that might help understand the intricate molecular mechanisms in human cancers.

Methods. Six CMT cell lines (CMT9, 12, 27, 28, 47 and 119) from different dog breeds, normal canine fibroblasts (NCF) and two canine melanoma cell lines (CML7C and 10P) were cultured and total RNA isolated by phenol chloroform extraction. The primers for p16 and other CKIs were designed by Vector NTI. The semi-quantitative (sq) RT-PCR and the PCR for rapid amplification of cDNA ends (RACE) were used to determine the sequence of p16 as well as expression of INK4 and CIP-KIP CKIs in all cell lines. The NCBI BLAST and Vector NTI sequence alignX tools were employed to identify canine p16 exons. The putatively translated protein structure was analyzed by protein motif/fold/structure prediction tools.

Results. From all PCR and subsequent cloning experiments, we found differential expression patterns for the CKI genes in CMT and CML cell lines. p16, p14ARF and p15 were not expressed in CMT12/27/119 cell lines. Similar expression defects were also present in CML cell lines. The complete p16 coding sequence was determined by overlapping PCR and aligned with p16 from other mammalian species. While a novel frameshift mutation was identified in p16 exon 1α of CMT28, a large deletion was discovered in p16 exon 1α form CML7C. Moreover the frameshift mutation in CMT28 was found to alter the entire reading frame from the exon 1 and 2 boundary and was predicted to disrupt the p16/p14 native protein folding and functional integrity.

Conclusions. Differential expression of the INK4 genes in our CMT/CML cell lines suggested that defects in these tumor suppressors frequently promote cell transformation in these cancers. The p16 gene defects mostly accumulated in exon 1α in both canine tumor models and this mapping strongly correlate to p16 mutations in human breast cancers. The alternative expression and frequent mutations in p16 from this study identify this tumor suppressor as a critical regulator and a potential therapeutic target in canine and human cancers.

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Flow Cytometric Analysis of Proliferative Responses of Chicken Peripheral Blood Mononuclear Cells Following Concanavalin A Stimulation

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Introduction: Radioactive, colorimetric, and single-cell assays are typically used for measuring peripheral blood mononuclear cell (PBMC) proliferative responses but they do not provide information regarding the number and phenotype of proliferating cells. To obtain a better understanding of proliferation of chicken PBMC subpopulations, we developed a carboxyfluorescein diacetate succinimidyl ester (CFSE)-based flow cytometry assay. The CFSE is an intracellular fluorescent dye that associates with intracellular proteins in a stable non-cytotoxic manner. This intracellular CFSE-staining combined with antibody-labeling of cell surface markers is used to measure proliferation of lymphocyte subpopulations in chicken PBMC following stimulation with concanavalin A (ConA), a classical T cell mitogen.

Methods: A study was conducted using 1 week old, naïve, specific pathogen-free (SPF) leghorn chicks. Chicks were housed at College of Veterinary Medicine, Auburn University. They were fed a conventional corn-soybean meal diet *ad libitum* and had free access to water. Intracellular staining of PBMC with CFSE was optimized by determining the CFSE concentration that allowed the detection of the maximum number of proliferation cycles over a 3-day period. Briefly, PBMCs were isolated by Histopaque Ficoll density gradient centrifugation and were stained for 15 min with increasing concentrations of CFSE [0 µM (unstained), 0.5 µM, 5 µM, 10 µM, or 25 µM]. The CFSE loaded cells were placed in a CO2 incubator after which the intracellular CFSE fluorescence intensity was measured at 19, 24, and 96 hours. Optimal staining was obtained when cells were stained with 10 µM CFSE for 15 min. Using this CFSE loading protocol, the proliferative response of CFSE-loaded PBMCs stimulated with ConA (10 µg / mL) over a 3-day period was evaluated. CFSE loaded PBMCs were labeled for T cell surface markers with anti-CD3, -CD4, and -CD8 antibodies. Controls for these procedures included cells not stained with CFSE, cells not stimulated with Con-A, and cells not labeled with antibodies. Flow cytometric analyses yielded mean fluorescence intensity values used to calculate the number of proliferations cycles of each cell. These data were used to calculate the proliferation indices induced by ConA, and to construct charts depicting the proportion of CD3+, CD4+, CD8+ proliferating cells.

Results: The 10 µM CFSE concentration was optimal for intracellular staining of chicken PBMCs for downstream flow cytometric analysis. Proliferation cycle charts showed that 42.1% of total PBMCs proliferated in response to stimulation with ConA. In addition, the proliferation index (PI) for ConA-stimulated cells was 0.57. T cell subpopulation analysis demonstrated that 85.5% of the cells were CD3+, 20.7% were CD4+, and 65.5% were CD8+. Thus, this assay allowed quantitative analysis of T cell subpopulations.

Conclusions: The CFSE-based flow cytometric assay developed was sensitive enough to determine the number of proliferating chicken PBMCs, the proliferation pattern of the PBMCs (number of proliferation cycles and proliferation indices), and the T cell subpopulations composition of proliferating cells when stimulated with ConA. As such, the developed assay could be a valuable tool for analyzing T cell responses to pathogens or vaccines.

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The Use of Phage Proteins Containing NGR-Motive for Targeted Delivery of Liposomal Doxorubicin (LipoDox) into Lung Cancer Cells

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Introduction: Lung cancer remains one of the leading causes of death in the United States. One of the common standards of care for the treatment of various types of cancer, including lung cancer, is doxorubicin. Doxorubicin exerts its cytotoxic effect on the cell by many proposed mechanisms; one mechanism is intercalation with the genomic DNA. However for this means of cell death to occur, doxorubicin must be delivered to the intracellular compartments and ultimately reach the nucleus to become effective. Here we use phage display technology to select phage fusion proteins that can be incorporated into liposomal nanomedicines. We hypothesize that the specific cell uptake properties of the selected phage proteins can be transferred to liposomal doxorubicin (LipoDox) by incorporation of the isolated proteins into the liposomal membrane of the drug carrier.

Methods: To develop ligands specific for a NSCLC cell line, we used a selection procedure using a landscape phage display library and were able to isolate two NGR containing phage fusion peptides. Phage were screened by binding affinity and specificity using previously described assays. Phage uptake was determined in target cells by incubating phage with target cells for 1 hour. Unbound phage were removed and phage were recovered by three different procedures: 1) surface bound phage were removed by acid treatment, 2) cytoplasmic phage were recovered by washing the cells after acid treatment, and 3) membrane associated phage were recovered by detergent lysis. The mechanism of endocytosis was studied by performing cell uptake experiments with different endocytosis inhibitors. Insertion of phage peptides into LipoDox was achieved using previously described methods and characterized for protein content, doxorubicin content, size distribution and zeta potential. Cytotoxicity of the preparations was determined by incubating cells with various concentrations of doxorubicin for 36 hours and measuring the cell viability after treatment. Cell viability was determined using an MTT assay following the manufacturer's instructions.

Results: Our results show that the two NGR containing phage bound specifically to Calu-3 cells. Phage uptake studies showed very different uptake profiles with phage clone ANGRPSMT showing equal distributions between cytoplasmic and membrane associated phage, while a phage clone VNGRAEAP showed almost an exclusive distribution on the cell surface and cytoplasmic fraction. Uptake studies using endocytic inhibitors showed these two peptides are internalized by two different mechanisms; phage displaying the peptide ANGRPSMT was internalized by clathrin- and caveolae-mediated pathways while phage displaying the VNGRAEAP peptide was shown to be internalized by caveolae-mediated endocytosis and macropinosytosis. Both ANGRPSMT and VNGRAEAP targeted LipoDox increased the efficacy of unmodified LipoDox in target Calu-3 NSCLC cells.

Conclusions: Phage display can produce ligands that are specific for NSCLC and these clones can be incorporated into liposomal nanomedicines to increase their efficiency to deliver their cargo to the intracellular compartments. We also show that similar motifs found within the selected phage peptides can modulate the endocytic pathway used for intracellular delivery which suggests that there may be two functional domains present in some of our selected phage fusion proteins.

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Oral Bacteriophage Treatment to Reduce Fecal Shedding of *Salmonella enterica* Serotype Newport from Calves

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Introduction. Antibiotic resistance in pathogenic bacteria is a growing problem in food-producing animals. In particular, *Salmonella enterica* subsp. *enterica* serotype Newport (*S.* Newport) strains are eighteen times more likely to be multi-drug resistant when obtained from bovine sources, and multi-drug resistant *Salmonella* strains have greater virulence when compared with drug-susceptible strains in humans. Thus, finding alternatives to antibiotic use is a critical need. Bacteriophage treatment has many advantages and oral administration of *Salmonella*-targeted bacteriophage was hypothesized to decrease the severity of clinical signs and the duration of fecal shedding.

Methods. Wild-type bacteriophages were obtained from *Salmonella* positive fecal samples and a cocktail containing five bacteriophages was designed. Treatment calves were administered 10^9 CFU's of *S.* Newport and subsequently treated with the bacteriophage cocktail (10^9 PFU's of each bacteriophage) when a fever spike was noted. Control calves were administered 10^9 CFU's of *S.* Newport, but were not treated with the bacteriophage cocktail.

Results. An immediate decrease in fecal *S.* Newport shedding was noted with a concomitant reduction in fever and a return to normal body temperatures after bacteriophage administration. On days 4-7 post inoculation, the mean $\log_{10}$ CFU's of *S.* Newport per gram of feces ranged from 0.425 to 2 in the treatment calves and was significantly lower than *S.* Newport shedding in the control calves (ranged from 3.9 to 6.3). On day 4 post inoculation, the mean temperature of the treatment calves was 102.5°F and was significantly lower than the mean temperature of the control calves (104.3°F). Bacterial cultures in the treatment calves became negative for *S*. Newport within 2-3 days of instituting bacteriophage treatment.

Conclusions. Treatment with bacteriophage is a viable alternative to antibiotic use in cattle, both for treatment and just before slaughter to decrease fecal contamination of meat by *Salmonella*. This would reduce the incidence of human infection obtained from bovine sources, decrease the use of antibiotics in cattle, and curtail the development of further antibiotic resistance in these isolates.

Acknowledgments. This project was supported by the Alabama Agricultural Experiment Station and by the Animal Health and Disease Research program in the College of Veterinary Medicine, Auburn University.

Potential anxiolytic mechanisms of *Scutellaria lateriflora* (American skullcap)

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Introduction. Anxiety is one of the most prevalent neuropsychological disorders worldwide. Findings of various researchers suggest roles of oxidative stress in neurodegenerative and neuropsychiatric diseases such as aggressiveness, depression and anxiety. Bioactive compounds present in medicinal plants neutralize or scavenge toxic free radicals thus suppress oxidative stress in cells. *Scutellaria lateriflora* (American skullcap), a native plant of North America, has been used by Americans and Europeans as a nerve tonic for more than 200 years. However, the neuroprotective effects of *Scutellaria lateriflora* are not fully understood. Therefore, the objective of this study is to investigate the antioxidant effects of *Scutellaria lateriflora* .

Methods. The antioxidant potential of aqueous or alcoholic extracts of *Scutellaria lateriflora* was determined in rat brain tissue using thiobarbituric acid reactive substances (TBARS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and dichlorodihydrofluorescein diacetate (DCF-DA) assay. 15-Lipoxegense inhibitory activity of ethanolic extract was examined using a Lipoxygenase Inhibitor Screening Assay Kit

Results. The antioxidant potential of aqueous or alcoholic extracts of *Scutellaria lateriflora* was determined in rat brain tissue. The ethanolic and aqueous extracts dose-dependently scavenged the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Only the ethanolic extract scavenged the tert-butyl peroxide-induced reactive oxygen species and reduced the lipid peroxidation in the mouse brain homogenate. The reduction of t-butyl peroxide-induced lipid peroxidation by ethanolic extract is positively correlated to its 15**-**lipoxygenase inhibitory activity. Furthermore, ethanolic extract of *Scutellaria lateriflora* protected hydrogen peroxide-UV induced cleavage of supercoiled and open circular plasmid DNA

Conclusions. In conclusion, due to the presence of polyphenols and flavonoids in *Scutellaria lateriflora* exhibits antioxidant activities and thereby conceivably prevents neuronal damage. Hence, *Scutellaria lateriflora* has good therapeutic potential in the future to treat patients suffering from anxiety disorders.

Protein Phosphatase PPM1A Positively Regulates Human Pregnane Xenobiotic Receptor-Mediated *CYP3A4* Gene Expression in HepG2 Human Liver Hepatocarcinoma Cells.

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Introduction: Cytochrome P450 3A4 (CYP3A4) is the most prevalent drug-metabolizing enzyme in human liver hepatocytes that catalyzes the metabolism of more than 50% of clinically used drugs. Human pregnane xenobiotic receptor (hPXR), a ligand-dependent transcription factor activated by a broad range of compounds including therapeutic drugs, plays a central role in activating the expression of CYP3A4. Expression of CYP3A4 is highly variable depending on the physiological or pathological status of liver. Changes in the expression of CYP3A4 can alter the therapeutic or toxicologic response to a drug and potentially lead to life-threatening adverse drug reactions. However, the molecular mechanisms that contribute to altered CYP3A4 expression are poorly understood. In the current study, we sought to determine whether Mg^{2+}/Mn^{2+}-dependent phosphatase 1A (PPM1A) regulates hPXR-mediated CYP3A4 expression in HepG2 human liver hepatocarcinoma cells.

Methods: Overexpression of hPXR and PPM1A was accomplished by transfecting the cells with the respective plasmids, whereas knockdown of endogenous PPM1A expression was achieved by transducing the cells with lentiviral particles carrying the specific shRNA. Transactivation assays and mammalian two-hybrid assays were performed to study *CYP3A4* promoter activity and hPXR- steroid receptor coactivator-1 (SRC-1) interaction, respectively.

Results: Overexpression of PPM1A significantly enhanced hPXR interaction with SRC-1 and hPXR-mediated *CYP3A4* promoter activity. In contrast, knockdown of endogenous PPM1A attenuated both hPXR interaction with SRC-1 and hPXR-mediated *CYP3A4* promoter activity.

Conclusions: These preliminary results implicate a novel role for PPM1A in regulating hPXR activity and CYP3A4 expression in liver hepatocytes. In addition, these observations suggest the hypothesis that deregulation of PPM1A expression may contribute to altered hPXR activity and CYP3A4 expression in hepatocytes.

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Determination of Virulence of Uropathogenic Canine *Escherichia coli*

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Introduction. *Escherichia coli* is the most common cause of urinary tract infections (UTI) in both human and small animals. Pathophysiology of UTI involves ascending translocation of microrganism from the GI tract. However, to translocate, these organisms must acquire virulence factor (VFs) genes that facilitate extraintestinal survival. Different VFs leads to differences in the extent and impact of infection and thus variable clinical signs, including lack of symptoms (asymptomatic bacteriuria [ABU]). Most evidence-based literature demonstrates that virulence and antimicrobial resistance genes do not generally co-habitate in the same isolate. However, increasing, in *E.* coli, co-habitation has been identified. Regardless, antimicrobial treatment of asymptomatic isolates may not be prudent: not only is the risk of multidrug antimicrobial resistance increased, but their removal may allow infection of the bladder with more pathogenic organisms. Our previous data suggests that up to 20% of UPEC associated with UTI in dogs or cats are asymptomatic bacteriuria (ABU). The goal of this study is to characterize uropathogens *E. coli* (UPEC) of dogs in order to identify unique genes that can be used to diagnose ABU and to identify an ABU that might be developed as a biotherapeutic alternative to antimicrobial therapy.
Methods. A total 68 canine uropathogen isolates were randomly selected from our library of 2659 canine and feline *E. coli* uropathogens. Isolates were subjected to multiplex PCR for characterization of the presence of 14 specific virulence genes commonly found in human UPEC associated with UTIs. 14 VF genes were categorized as to adhesins (*papG, papA, papC, focG, iha, fimH, sfaS,* and *focA*), toxins (*hlyD, sat,* and *cnf1*), or iron acquisition proteins (*iut, iroN*, and *ireA*). Each isolate was categorized as to its association with clinical signs ranging in severity from absent (n=15), mild (n=18), moderate (n=15), severe (n=17) to life-threatening (n=3). Each isolate was also designated as ABU (severity absent) or non-ABU (severity levels 2-5). Proportional comparisons were made with regard to virulence profiles, stratified by severity of clinical signs associated with the infections. Expression of VF genes determined by *q*RT-PCR; levels of expression was compared to *gapA* as a housekeeping gene and normalized to the expression in *E. coli* ATCC 25922.
Results. Of the 68 UPEC isolates, 15 (22%) were ABU (n=15) while 43 (78%)were non-ABU. While the proportion of each of the 14 virulence genes did not different among the 5 levels of severity (*P>0.05)*, significant differences did occur in the proportion of isolates categorized as ABU vs non-ABU. These included: *papG, focG, cnf1, ireA* and *sfaS* (*P<0.05*). The largest discriminator between ABU and non-ABU appeared to be *cnf1* (47% VS 68%; *P<0.05*).
Conclusions. Virulence profiles of clinical canine UPEC do differ significantly between ABU and non-ABU. These results suggest that further regression analysis of the presence of VF such that a VF profile can be identified may help in the pre-treatment identification of ABU *E. coli* such that a “no-treatment” option might be considered. Further, such isolates might be developed as a therapy alternative to antimicrobials. Both goals will support de-escalation of antimicrobial use and thus antimicrobial resistance.

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Significant Therapeutic Benefit after Postsymptomatic Gene Therapy in a Feline Model of Sandhoff Disease

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Introduction. Lysosomal hexosaminidase A (HexA) deficiency causes neuronal storage of GM2 ganglioside, resulting in progressive neurological deterioration and death, often in infancy. AAV gene therapy has been extraordinarily successful in the GM2 mouse model (*Proc Natl Acad Sci USA*, 103:10373, 2006) and GM2 feline model (unpublished), which has a brain size and complexity more closely resembling humans. Eight GM2 cats treated before disease onset reached humane endpoint between 9.8-29.9 months old and another AAV-treated GM2 cat is currently 28.8 months old (untreated humane endpoint, 4.4 ±0.6 months, n=9). Because most humans are not diagnosed until disease symptoms become evident, it is imperative to test AAV gene therapy in post-symptomatic GM2 cats more representative of human patients in future clinical trials.

Methods. Monocistronic AAV2/rh8 vectors expressing feline Hex α and β subunit cDNAs (2 e12 g.c. per vector) were injected bilaterally into the thalamus and deep cerebellar nuclei of early post-symptomatic (1.6-1.8 months old, n=4) or late post-symptomatic (2.1 and 2.7 months old) GM2 cats (disease onset 1.4 ±0.4 months). Clinical therapeutic outcome was evaluated by (1) a clinical rating scale reflecting the neurodegenerative course of GM2 untreated cats and (2) lifespan. Brain and spinal cord were collected at necropsy and analyzed for HexA distribution.

Results. The early post-symptomatic group reached humane endpoint between 10.6-18.9 months old. HexA activity was present at near or above normal levels throughout the brain (1.1-22.4 fold normal) and spinal cord (0.8-4.1), and PAS staining showed reduced ganglioside storage in many areas of the treated CNS. The late post-symptomatic group did not show any clinical improvement over untreated GM2 cats. HexA activity was restored to 0.4-14.5 fold normal in the brain and 0.3-3.1 fold normal in the spinal cord, which is above the hypothesized therapeutic threshold (>0.1 fold normal), but average HexA activities were lower than those in the early post-symptomatic group. The late post-symptomatic group reached humane endpoint according to stereotypical disease progression in untreated GM2 cats, which consists of debilitating whole body tremors and severe balance disturbances. In contrast, and similar to pre-symptomatic AAV-treated cats, the early post-symptomatic group lost the ability to stand due to hind limb weakness, with or without joint abnormalities, but debilitating tremors or balance problems either did not develop or improved.

Conclusions. AAV gene therapy can be beneficial if administered after the onset of clinical disease symptoms, which is when most human patients are diagnosed. However, our results also suggest that there is a point in disease progression beyond which AAV gene therapy is of no therapeutic benefit. These translational studies provide strong support for the initiation of AAV-based clinical trials for human GM2 gangliosidosis.

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Undergraduate Student Poster Presentations

Towards developing a PNP Activated Therapy for Canine Cancer

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Introduction. Enzyme-activating prodrug therapy has been used as an approach in the treatment of solid tumors. We propose to demonstrate that expression of *Escherichia coli* purine nucleoside phosphorylase (PNP) by cancer cells results in increased cell death in the presence of the prodrug 6-methylpurine-2'-deoxyriboside (MeP-dR). *E. coli* PNP is a homohexameric enzyme consisting of a molecular weight of approximately 150 kDa. Following delivery to target cells, *E. coli* PNP has previously been shown to generate toxic purine bases from a prodrug substrate that inhibits protein synthesis, DNA synthesis and RNA synthesis and therefore can kill both dividing and non-dividing cells. A high bystander effect is seen among neighboring cells due to the toxic drug's ability to cross cell membranes. In this gene therapy approach we used a recombinant adenoviral vector containing the PNP gene, Ad5PNP, to transfect target cells and permit expression of the enzyme by the canine cells in vitro. We then analyzed the ability of PNP to increase the toxic effects of MeP-dR on canine cancer cell lines.

Methods. Three canine melanoma lines (CML2, CML10 andCML27), two mammary tumor (CMT27, CMT28) and a histiocytoma (DH82) cell line were used for all experiments. To assess the effect of MeP-dR alone, cells were incubated in the presence of the prodrug at a range of concentrations for 96 hours and cell viability measured. To assess the effect of MeP-dR in the presence of PNP, cells were transfected with AdPNP at different multiplicities of infection (moi), and then treated with a range of concentrations of MeP-dR and incubated at 37^{o}C for 96 hours. Cell viability was determined using a Cyquant cell viability assay.

Results. In the presence of MePdR alone, there was no effect on cell viability on most of the cell lines even at concentrations as high as 500uM. There was a modest effect seen in the DH82 cell line. In cells transfected with PNP, cell viability was reduced in all cell lines in the presence of both PNP and MeP-dR. Cell viability decreased with increased virus particles and increased concentration of MeP-dR.

Conclusions. We have demonstrated that treatment with MeP-dR on canine melanoma, mammary tumor and histiocytoma cell lines transfected with the PNP gene showed reduced cell viability. These results indicate that AdPNP in conjunction with MeP-dR resulted in reduced cell viability, when compared to treatment with MeP-dR alone, in these cell lines. Therefore the use of AdPNP mediated prodrug therapy may be a potential useful therapy for the treatment of canine cancers.

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Evaluation of *sdiA* Expression in *Escherichia Coli* on Antimicrobial Resistance

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Introduction. The purpose of this study is to determine the effect of *sdiA* gene mutation in Non-Drug-Resistant (NDR), Single-Drug-Resistant (SDR), and Multiple-Drug-Resistant (MDR) clinical *Escherichia coli* isolates. The *sdiA* gene plays an important regulatory role in the quorum sensing, or cell communication, abilities of *E.coli*. *SdiA* regulates the secretion of signaling molecules that bind to the receptors on other microorganisms and results in that microorganism releasing its own signaling molecules. The density of the microorganisms can then be determined by the concentration of signaling proteins. If the size of the inoculum of microorganisms in an area is high enough, the microorganisms can then act as a single organism, becoming both more efficient and pathogenic by forming a biofilm, using efflux pumps, or producing spores. In *E.coli* specifically, the use of the quorum sensing gene *sdiA* may play a role in drug resistance by stimulating efflux pumps to rid the organism of the drugs. Because these genes coincide with increased drug resistance, it is hypothesized that overexpression of *sdiA* will be higher in MDR organisms than in NDR and SDR organisms.

Methods. Five isolates that were previously identified as NDR, nine isolates previously identified as SDR, MDR, and MDR-FQ, each, were selected and grown overnight. Inocula were prepared by suspending growth from the overnight cultures in sterile saline to a turbidity of approximately 0.5 McFarland turbidity standard. Final inocula contained 2 to 7x105 CFU/ml. The suspension was used to inoculate LB broth and isolates were incubated at 37°C for three hours. The RNA of each isolate was isolated using Quiagen's protocol and subjected to reverse transcription to make cDNA. RT-PCR was run on the cDNA of each sample, using *Gap A* as a housekeeping gene. The level of expression was normalized with *E. coli* ATCC 25922 and those with expression over 2.0 were considered to have overexpression of the *sdiA* gene.

Results. Overexpression was found in 2 isolates and now larger groups of samples are being processed in order to determine specific patterns of *sdiA* gene expression.

Conclusions. At the present time, there are no definitive conclusions to report.

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Precision of the Neubauer Hemocytometer in Quantifying Spermatozoal Concentration: Does the Experience Level of the Operator Affect Results?

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Introduction. For quantifying the concentration of spermatozoal samples, the Neubauer hemocytometer has long been considered the gold standard by the World Health Organization (WHO). The hemocytometer is relatively simple to use and cost-effective. However, it is time-consuming compared to other methods of quantification. In this study, we estimated the standard deviation (i.e., precision) in estimates of sperm concentration between the two chambers of the hemocytometer and between replicate samples from a given ejaculate when run by an experienced operator versus an inexperienced one. With this information, we determined whether or not the degree of operator experience would significantly affect the results of the hemocytometer.

Methods. Each ejaculate was analyzed a total of four times: twice by an experienced person and twice by an inexperienced one. Thus, a mean concentration for each ejaculate was obtained by each person. The standard deviation in concentration between replicate samples for each person and for a given ejaculate was calculated as an estimate of inter-replicate precision. Similarly, the standard deviation in concentration between chambers from each hemocytometer was calculated as an estimate of inter-chamber precision. One hundred different samples (ejaculates) were obtained from a total of nineteen different dogs. The dog breeds used were Labrador Retrievers (8), Beagles (1), English Pointers (2), and Beagle-Corgi crosses (7). Dogs ranged from one to seven years of age with a mean of 4 years of age.

Results. Standard deviation in estimated sperm count between the two chambers of the hemocytometer was estimated to be 1.97 (± 2.03; ± 95% C.I.) million sperm/ml higher for the inexperienced operator relative to the experienced operator; however, differences were not statistically significant at the $\alpha = 0.05$ level ($t_{281} = 1.90$, $p = 0.058$). Standard deviation in estimated sperm count between replicate slides of the hemocytometer was estimated to be 2.80 (± 1.96; ± 95% C.I.) million sperm/ml higher for the inexperienced operator relative to the experienced operator; however, differences were not statistically significant ($t_{99} = 1.43$, $p = 0.1562$).

Conclusions. There was no statistically-significant difference between the precision of either inter-chamber counts or inter-replicate counts when run by an experienced person versus an inexperienced person. This is important because it tells us that the hemocytometer can be used by a properly trained, yet inexperienced person in a clinical setting while still obtaining results with the same precision as the veterinarian.

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Lung Cancer Specific Phage Fusion Protein Modulates the Cytotoxicity of Liposomal Doxorubicin (LipoDox)

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Introduction: Lung cancer still remains one of the leading causes of death in the United States. Doxorubicin is a common standard of care for the treatment of various types of cancer with liposomal doxorubicin products (LipoDox/Caelyx/Doxil) being nanomedicines currently used that provide decreased cytotoxicity and an increased circulation time in the body and increased accumulation at the tumor site. We have previously demonstrated in a breast cancer model that targeted doxorubicin provides an additional increase of doxorubicin accumulated inside target cells. Using a phage display library produced in our lab, we were able to select for a lung cancer binding phage that interacted specifically to our target cell line Calu-3. We hypothesized that the specific binding of phage to their target could be translated to an increased binding of targeted liposomes and increase the intracellular levels of cytotoxic drug.

Methods: Phage clone ANDVYLD was selected from a biopanning experiment to a NSCLC cell line and isolated for further study. Phage major coat protein was isolated from phage by standard size exclusion chromatography in 10 mM cholate buffer. Isolated major coat protein was then inserted into LipoDox at a rate of 0.5% v/v protein/lipid content by incubation in 15 mM cholate buffer at 37ºC overnight. Cholate was removed by dialysis in decreasing concentrations of cholate. Samples were then purified by size exclusion chromatography to remove unencapsuated doxorubicin and unincorporated protein and concentrated with 100K MWCO concentration filters. Phage protein concentration and orientation was determined by treatment with proteinase K and detecting for presence of phage protein by Western blotting. Doxorubicin was freed by lysis of liposomes with 1% v/v Triton X-100 and quantified using UV/Vis spectroscopy. Liposomes were assayed for size distribution and zeta potential using DLS with a Malvern ZetaSizer Nano. Calu-3 cells were plated at a density of 5,000 cells/well and incubated with various concentrations of phage-LipoDox for 24 hours in a 37ºC/5% CO_2 incubator. Cell viability was assessed by MTT assay according to the manufacturer's recommendations.

Results: Phage ANDVYLD was shown to bind selectively to Calu-3 cells and not to normal small airway epithelial cells. Physical characterization of ANDVYLD-LipoDox liposomes shows that phage protein is incorporated into the liposomes in the correct orientation with the N-terminus exposed to solution and the C-terminus remaining after proteinase K degradation. Modification of LipoDox did not produce any significant increases in the size distribution or zeta potential. ANDVYLD-LipoDox was able to decrease the number of viable cells after 24 hours of treatment compared to unmodified LipoDox.

Conclusions: Phage derived major coat proteins provide a means to specifically target liposomal nanomedicines specifically to target cells. Here we show that ANDVYLD-LipoDox preparations can effectively decrease the required dose to achieve a lethal dosage of doxorubicin in Calu-3 cells. Our results show that targeting of LipoDox can increase the intracellular accumulation of doxorubicin which leads to a decreased number of viable cells after treatment and a subsequent bypass of the multidrug resistance phenotype observed in Calu-3 cells.

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The Variation of Articular Cartilage Stiffness among Diverse Equine Joint Types

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Introduction. Because cartilage has no blood flow, the recovery from injury is slow at best. Damaged cartilage is replaced with fibrocartilage, which is mechanically much different from normal articular cartilage, thus injury leads to a need for repair or replacement with an artificial joint. Currently, most artificial joints, which only last around 20 years, are designed by choosing a combination of hard surfaces that reduce the amount of wear, unlike synovial joints which have a softer layer of articular cartilage covering opposing surfaces of subchondral bone within the joint(4). Since cartilage is a self-adapting material, the purpose of this work is to characterize how cartilage might be adapted for different loads.

Methods. The experimental population contained the following number of cadaveric equine joints: 10 fetlocks, 5 stifles and 9 carpi. Articular surfaces of interest were medial and lateral condyles of distal metacarpal III, medial femoral condyle, and medial aspects of distal radius, proximal radial carpal bone, distal radial carpal bone and proximal third carpal bone within the carpus. Samples were collected, dissected and tested within 24 hours of the time of death. All tests were performed in a 0.9% NaCl bath to prevent desiccation of the articular cartilage. Using a CETR UMT-3 tribometer, indention tests were performed where a 10mm circular punch compressed the cartilage surface at a constant velocity, allowing the UMT-3 to record the contact force. The stiffness of the cartilage was then calculated as the instantaneous gradient of the force as a function of displacement. Tests were repeated multiple times on each surface to increase the reliability of the data collected.

Results. The fetlock (MCIII) showed the greatest contact force, with the averaged aforementioned carpal surfaces and then the medial condyle of the stifle showing lower forces, in that order. The stiffness also followed a similar trend and also varied with deformation, displaying the well characterized viscoelastic behavior of cartilage.

Conclusions. The indentation tests performed in this work suggests that different equine joint surfaces have different stiffness and therefore different mechanical properties.

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Liposomal Doxorubicin Targeted by Fusion Phage Protein

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Introduction. Specific targeting of nanomedicines has long been a major goal for the improvement of anticancer treatments. Phage display technologies may hold the answer to achieving such an improvement. The tumor-specific peptides, genetically fused to all 4,000 copies of the phage's major coat protein, can be affinity selected from multibillion clone libraries by their ability to bind specifically to cancer cells, penetrate into the cells, or accumulate in the tumor-surrounding vasculature. The selected phages can be converted into drug-loaded liposomes and micelles, in which the fusion phage proteins span the lipid membrane and display the tumor-binding peptides on the surface of the vesicles. This offers the advantage of enhanced binding and killing of tumor cells. We therefore tested the pancreatic carcinoma selected phage (ETPPSWGGG) modified Lipodox to evaluate its increased binding and cytotoxicity towards target PANC-1 cells in comparison with non-cancerous cells.

Methods. We propagated cultures of the PANC-1 specific ETPPSWGGG phage to harvest the major coat proteins which were then inserted into Lipodox that had been destabilized in 200 mM cholate. After removal of the cholate via gradual dialysis, the proteins stabilized in an orientation with the polar N-terminus (receptor end) facing extracellular space, which allows interaction with Panc-1. An MTT assay was then performed for ETPPSWGGG modified Lipodox, unmodified Lipodox, and doxorubicin activity against Panc-1 to determine the extent of cytotoxic effect.

Results. Lipodox modified with phage fusion protein affected its cytotoxic activity.

Conclusions This study implies that phage technology can be used in an inexpensive and efficient way to increase the targeting of Liposomal Doxorubicin.

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Identification of cell compartment-specific gene expression events associated with estrogen receptor-alpha dependent endometrial development using multispectral imaging and digital image processing

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Introduction. In the pig (*Sus scrofa domesticus*), as in other mammals, uterine development is incomplete at birth (postnatal day = PND 0) and continues postnatally with formation of endometrial glands, or adenogenesis. The uterine endometrium is composed of three cell types including luminal epithelium (LE), glandular epithelium (GE), and stroma (ST). Supported by stromal-epithelial interactions, adenogenesis occurs as GE differentiates from LE and proliferates into underlying stroma. Porcine adenogenesis is an estrogen-sensitive, estrogen receptor-alpha (ESR1) -dependent process initiated by PND 1. Disruption of adenogenesis can compromise adult uterine function. Understanding endometrial cell compartment-specific ESR1 expression will provide insight into mechanisms driving adenogenesis.

Methods. Expression of ESR1 can be quantified *in situ* using immunofluorescence (IF), multispectral imaging (MSI) and digital image processing (DIP). Here, objectives were to establish a protocol for automated: (1) DIP-based identification of endometrial cell compartments (GE, LE and ST) using cytokeratin-8 (CK8) as a marker of epithelium (LE + GE) and ESR1 as a specific marker of nascent GE; and (2) evaluation of cell compartment-specific ESR1 expression. To eliminate animal variation uterine tissue sections generated from one PND 5 gilt were fluorescently labeled for ESR1, CK8 and stained for cell nuclei. Images, obtained by MSI, were analyzed using CellProfiler™ and CellProfiler™ Analyst (www.cellprofiler.com). Results were compared to those obtained using manual cell compartment identification. Quantitative data for ESR1 expression and labeling index (percent ESR1-positive cells in each compartment) were subjected to analyses of variance.

Results. Procedures for automated identification of endometrial cell compartments (GE, LE and ST) and capture of quantitative cell compartment-specific data for ESR1 expression were established. Using automated procedures, data were obtained for 5,630 cells in all compartments. For both manual and automated methods, ESR1 expression was higher ($P < 0.001$) in epithelium than in stroma and, for epithelium, both ESR1 expression and labeling index were higher ($P < 0.001$) in GE than in LE.

Conclusions. Procedures established here permit automated assessment of specific cell characteristics *in situ*. This is important because cells removed from context in primary culture rarely behave as they would be expected to *in vivo*. With a novel protocol for automated, DIP-based evaluation of cell compartment-specific events associated with endometrial development now in hand, the stage is set to pursue investigations of cell-cell interactions essential for support of uterine adenogenesis.

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Diagnostic PCR-based Assay for Feline GM2 Gangliosidosis

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Introduction. Lysosomal storage diseases result from the dysfunction of lysosomal hydrolases responsible for degrading complex cell constituents, thus leading to abnormal accumulation of undegraded substrates. A type of lysosomal storage disease, GM2 gangliosidosis results in the buildup of GM2 ganglioside due to the absence of its hydrolytic enzyme, hexosamindase. Children with GM2 gangliosidosis die by 5 years of age, usually after years spent in a semi-vegetative state. The feline GM2 model is essential for development of therapies to treat this otherwise fatal disorder. Accurate, cost-effective and rapid genotypic diagnosis of GM2 cats is crucial to optimizing future therapies for gangliosidosis. Currently, the diagnostic procedure is dependent upon automated fluorescent DNA sequencing from a core facility, which is slower and more costly than assays that can be performed solely in-house. In addition, each step in the diagnostic process introduces additional opportunities for human error. Therefore, our goal is to develop a 2-step, in-house diagnostic assay that relies solely on PCR and gel electrophoresis.

Methods. The feline GM2 mutation is a 25 base pair inversion which has been exploited to design a PCR primer that can function in both coding and non-coding strand directions. Two additional primers that flank the mutation site allow genotypic identification by a simple PCR reaction and gel electrophoresis. Genomic DNA template for the assay may be obtained from blood, cheek swabs or any appropriate tissue. Standard PCR reaction components and methodology are used.

Results. Appropriate primer combinations generated 3 amplicons (500, 350 and 200 base pairs) that were easily differentiated on an agarose gel to identify the following genotypes: GM2 affected (500+350 bp), Wild-type (350+200 bp) and Heterozygote (500+350+200 bp). Validation and reproducibility of results will be established by automated fluorescent DNA sequencing.

Conclusions. These results will dramatically streamline our laboratory's ability to diagnose affected animals in a reliable, timely fashion, thereby enhancing the efficiency and design of therapeutic experiments. Results of ongoing and future translational studies using adeno-associated virus gene therapy are expected to underpin a human clinical trial for GM2 gangliosidosis in the near future.

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IBV- Ab and S1 Spike Protein Dominant B cell Epitopes Induced After Ocular Immunization with Ad5-S1.

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Introduction. Infectious Bronchitis Virus (IBV) causes considerable economic losses in the poultry industry worldwide. The transmembrane spike (S) glycoprotein of IBV includes a large external domain (with two subdomains), the transmembrane sequence and a small internal domain. The highly variable external spike protein provides this virus its antigenic properties, contains the host attachment site, and is the main target for immune responses (Cavanagh, 1983 and 1986). We have developed a replication-defective recombinant adenovirus expressing the amino terminal portion (S1) of IBV S gene (Ad5-S1). *Our goal was to determine the ability of Ad5-S1 to induce antibody (Ab) responses and determine dominant B cell epitopes on the S1 spike protein*.
Methods. Ten day old specific pathogen free chickens were either ocular or intramuscular immunized with 100µl PBS containing $9.3x10^7$ infectious units of Ad5-S1 or maintained as unvaccinated controls. The chickens were boosted 3 weeks post priming. Plasma and peripheral blood mononuclear cells (PBMCs) were collected 10 days after the boost. Lymphocytes were stimulated with 10µg/ml of lipopolysaccharide (LPS). Plasma and culture supernatants were stored at 4°C until enzyme-linked immunosorbent assays (ELISAs) were performed. Briefly, ELISA plates were coated with 5µg/ml of heat-killed IBV in carbonate buffer, pH 9.4. The plates were blocked, after which 2-fold dilutions of anti-sera were loaded, and Ab binding was detected using anti-chicken specific IgG or IgA biotinylated Abs followed by incubation with streptavidin-horse radish peroxidase (HRP) conjugate and addition of substrate for 30 minutes. Plasma samples were analyzed for reactivity on a peptide array consisting of 75 peptides of 17 amino acids long peptides with a 10 amino acid overlap covering the entire 540 amino acid long S1 sequence in order to determine the location of dominant linear B cell epitopes. Peptide ELISAs were performed as described above except that plates were coated with peptides (3 µg/ml) an plasma samples were used at 50 fold dilution. PBMCs were isolated and analyzed by flow cytometry by measuring binding of biotinylated IBV to B cells followed by staining with streptavidin –Alexa660.
Results. Both IBV-specific IgA and IgG levels in plasma increased compared to naïve controls ~6-weeks after ocular or intramuscular Ad5-S1 immunization. This immunization also caused an increase of IBV-specific B cells in the blood. PBMCs from vaccinated birds stimulated *in vitro* with LPS significant increased IBV-specific IgA and IgG Abs in the culture supernatant over levels in culture supernatant from unvaccinated controls. Measuring the ability of plasma Abs to react with the peptide array covering the S1 sequence the plasma were divided in low, medium and high responders. These 3 groups demonstrated that IgA Abs reacted with 5-8 dominant B cells epitopes. The epitopes increased with lower IBV plasma titers. Thus lower responders to IBV had a less focused humoral response to S1. Two to three of these epitopes were located in the S1 host attachment site, indicating these may be neutralizing Abs. The IgG plasma Abs also reacted with 1-5 S1-based B cell epitopes of which 1 was identical to those observed by IgA Abs and reacted with the host attachment site of S1. The IgG Abs of high IBV plasma responders reacted with all 75 peptides, while the low and medium group had 2 and 5 dominant B cell epitopes, respectively.
Conclusions. The Ad5-S1 vector is able to induce IBV-specific humoral immunity after ocular or intramuscular immunization. The magnitude of these responses was low due to the low titer of Ad5-S1. Based on induction of Abs to linear B cell epitopes in the host attachment site, this vector may induce IBV neutralizing Abs. Furthermore, chickens with high responses to IBV contained highly cross-reactive Abs to these IBV S1-based peptides after Ad5-S1 immunization.
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Comparison of complete genome sequences of ArkDPI-derived infectious bronchitis virus vaccine before and after a single passage in chickens

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Introduction. The ArkDPI molecular type of avian infectious bronchitis virus (IBV) is frequently isolated from commercial chickens in the southeastern US, despite the use of ArkDPI-derived vaccines. Live-attenuated IBV vaccines are attenuated by serial passage in chicken embryos. Based on spike (S) gene sequences of vaccine virus in vaccinated chickens, we previously found that specific vaccine subpopulations are rapidly selected in chickens from all four commercial ArkDPI-derived vaccines. This work focuses on one of the vaccines, from which subpopulations selected in different individual chickens all had the same S1 sequence. We determined the sequence of the entire genome of the vaccine virus prior to inoculation into chickens and vaccine virus in a pool of tears collected from chickens inoculated with that same vaccine in order to identify genomic differences outside the S gene. Each sequence was compared to a virulent, lower embryo passage of the Ark-DPI isolate, passage (p)11 and a more attenuated version of that same virus (p101). Others have shown that p11 and p101 differ in only 21 nucleotides (9 nucleotides in the S gene and 12 nucleotides outside the S gene). Our attention was focused on these 9 nucleotides while also analyzing the entire genome for additional potential differences. These changes outside the S gene produced as the vaccine underwent selective pressures in the host are crucial because they highlight locations in addition to the S gene that may be important in the fitness of the virus. Our goal is to determine the role of these variable nucleotides in the attenuation of the virus.

Methods. Sequences were generated using viral RNA prepared from the vaccine and viral RNA prepared from chickens 5 days after vaccination, from tear samples from 10 individual vaccinated chickens and a pool of tears from vaccinated chickens collected in an independent experiment. cDNA was synthesized by RT-PCR and submitted for sequencing. Sequences were assembled with those of p11 and p101 using MacVector. The pre-inoculation sequence of the virus was then compared to the post-inoculation sequences to identify nucleotide differences between the two.

Results. Among the 12 positions outside the S gene where differences were noted between p11 and p101, 4 positions were noted where a heterogeneous position in the vaccine genome became homogenous after passage in chickens. The vaccine genome was heterogeneous at 5 additional positions outside the S gene where differences had not been noted between p11 and p101. At 3 of these positions the vaccine genome became more homogenous after passage in chickens.

Conclusions. At 3 of the 4 heterogeneous positions in the vaccine genome where differences had been identified between p11 and p101, after passage in chickens the vaccine genome became predominantly like that of the virulent low passage (p11) virus. All but one change caused an amino acid change. This means that each of these changes has the potential to contribute to the fitness and/or attenuation of IBV.

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Post-Graduate / Faculty Poster Presentations

Evaluation of digoxin as an internal standard for analysis of bioactive compound [astragaloside IV] in medicinal plant *A. membranaceus* using High Performance Liquid Chromatography-Evaporative Light-Scattering Detection (HPLC-ELSD)

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Introduction. The use of medicinal plants to treat disease dates back to Mesopotamia during 2006 BC. Today, medicinal plants and other forms of alternative medicine are combined with modern medicine to provide a holistic medical approach. One of the ultimate goals of this research program is to identify specific herbs and cultivation conditions that will provide alternative crops that are well-adapted to the environmental conditions in Alabama. The optimal cultivation methods are determined by measuring effects on plant growth and production of bioactive compounds. *Astragalus membranaceus* produces astragaloside IV which supports immune function, and has anti-hypertensive, anti-inflammatory, hepatoprotective, and neuroprotective effects. HPLC-ELSD has been used to measure astragalosides because they have - weak chromophoric group in the UV region. This is the first report to identify an internal standard (digoxin) that can be used to potentially increase the accurate measurement of extraction efficiency of each sample.

Methods. The chromatographic method developed in this research was adapted and modified from Ganzera, Bedir, Calis, and Khan, 2001; and Li and Fitzloff, 2001. A Waters^{R} HPLC system and Sedex 75 ELS detector were used. A gradient mobile phase [acetonitrile (A) and Water (B)], with run time of 45 minutes was used. The calibration curve was prepared with astragaloside IV standard (ChromaDex) and digoxin (Sigma-Aldrich) internal standard. The calibration curve was calculated as peak area ratio (peak area ratio equaled peak area of astragaloside IV divided by peak area of digoxin) versus the concentration of astragaloside IV. The *A. membranaceus* roots were dried, ground to a powder, and extracted with methanol. Validation included measurement of precision by intra-and interday quantitations.

Results. The linearity of the calibration curve over the range of 10 to 80 ug/ml, was R^2=0.9932, and the elution times for digoxin and astragaloside IV were 9.94 and 23.08 minutes. The Limit of Detection and Limit of Quantitation were 60 ng and 120 ng. Recovery of astragaloside IV averaged 93%. Intra- and interday reproducibility, expressed as percent relative standard deviation (standard deviation divided by mean, multiplied by 100), and was less than 11.21%, 4.69%, and 2.97% for 10, 20, and 30 ug/ml spiked samples, respectively.

Conclusions. Digoxin's use as an internal standard is justified by linearity of calibration curves, good recovery, and sensitive Limit of Detection, similar to published reports. Its use is warranted in cases of small and varied extraction efficiencies.

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MRI and MRS of AAV treated feline and ovine models of Gm2 gangliosidosis

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Introduction. Sandhoff (SD) and Tay Sachs disease (TSD) are forms of GM2 gangliosidosis in humans that are untreatable and fatal by the age of 5 years in humans. They are caused by mutations of either the α subunit (Tay Sachs disease) or the β subunit (Sandhoff disease; SD) of the dimeric enzyme hexosaminidase. We performed intracranial (IC) AAV mediated hexosaminidase gene replacement in Jacob sheep (α subunit deficient) and GM2 cats (β subunit deficient). Results in Sandhoff cats show a >four-fold increase in lifespan, with marked attenuation of neurologic signs. With this profound success in IC therapy, human clinical trials are in the planning stages and development of non-invasive methods to evaluate amelioration or disease progression are vital to predicting outcomes in humans. Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are modalities that provide *in vivo* structural and biochemical information about the brain.

Methods. Monocistronic AAVrh8 vectors expressing feline Hex α and β subunits (1:1 ratio) were injected bilaterally into the thalamus and deep cerebellar nuclei of Sandhoff cats (4.4×10^{12} g.c. total). AAVrh8 bicistronic vector expressing ovine Hex α and β subunits was injected bilaterally into the thalamus (1.54×10^{12} gc/vector) and left lateral ventricle (4.2×10^{12} gc/vector). MRI and MRS analysis were performed using 3 Tesla (T) Siemens Verio open-bore MRI scanner at 4 months and 24 months for cats and ~11 months for sheep.

Results. MRI demonstrates that cortical white matter is hyperintense to gray matter at the level of the thalamus in an untreated SD cat (4 mos.) and TSD Sheep (10.4mos) just prior to humane endpoint. The AAV-treated SD cat (24mos) and TS sheep (11mos) have normalized white:gray matter intensities. Similarly, the deep cerebellar nuclei of the untreated SD cat are hyperintense relative to the cerebellar vermis, while DCN are hypointense in a normal cat and an AAV-treated SD cat at 24 mos of age. MRS revealed increased ratios of N-acetyl-aspartate (NAA)/Creatine (Cr) (4.26) in untreated SD cats near the humane endpoint (4 mos.) compared to normal (NAA/Cr 1.64). At 24 mo. old, an AAV-treated SD cat showed normalized NAA/Cr (1.62). MRS of TSD sheep shows decreased NAA/Cr (0.95) and increased MI/Cr (0.93) in the old untreated TSD sheep as compared to normal (NAA/Cr 2.09 MI/Cr 0.49). The AAV treated TSD sheep showed NAA/Cr (1.40) and MI/Cr (0.76) levels intermediate to normal and untreated. Voxel placement was consistent between sheep and cats and was located in the parietal cortex.

Conclusions. Here we show 3T MRI images in cats and sheep showing preservation of white and grey matter structures as well as MRS data showing normalization of brain metabolites.

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Transabdominal Ultrasound of Adrenal Glands in Horses

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Introduction. Previous reports have identified adrenal glands in horses using both transrectal and laproscopic procedures. A pilot study in 2009 at Auburn University, using four different ultrasonographers, examined the feasibility of identifying the adrenal glands via transabdominal ultrasound in 11 horses. In the previous study both adrenal glands were identified and measured in 10/11 horses using a transabdominal ultrasound technique. The purpose of this study was to refine the technique for localization and measurement of the adrenal glands in 10 horses using one ultrasonographer.

Methods. Ten adult horses were used without fasting prior to the examination. The hair was clipped then alcohol and ultrasound gel was applied to the skin. Minimal restraint and sedation as needed was used during the ultrasound examination. Transabdominal ultrasound was performed using a C4-2 curvilinear probe or a S3-1 sector array probe. A sagittal imaging plane was used for evaluation of all adrenal glands imaging just cranial to the renal artery as it leaves the hilus of the kidney. The right kidney was located beginning at the 16th intercostal space, the right adrenal gland was located cranial and medial to the kidney and lateral to the caudal vena cava. The left kidney lies medial to the spleen beginning at the 17th intercostal space, the left adrenal gland was located craniomedial to the kidney.

Results. Both right and left adrenal glands were identified in all 10 horses. In three horses the ultrasound scans had to be repeated 4-6 hours later due to intestinal gas obscuring visualization of the kidneys and adrenal glands. The adrenal glands appeared hypoechoic relative to the kidney and surrounding fat. The scanning depth for location of the right adrenal gland was 15.5 ± 1.42 cm. The right adrenal gland length was 4.41 ± .75 cm and the width 1.17 ± .24 cm. The scanning depth for the left adrenal gland was 19.2 ± 2.29 cm. The left adrenal length was 4.6 ± .82 cm and width 1.08 ± .26 cm.

Conclusions. Transabdominal ultrasound is a reliable method for evaluation of adrenal glands in adult horses. Repeat imaging in 4-6 hours may be needed if the patient has not been fasted prior to the ultrasound examination.

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Adverse effects of rotenone-induced mitochondrial complex I inhibition in mouse hippocampus are reversed with antioxidant treatment

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Introduction. Neurons, with their high basal and activity-dependent metabolic energy needs, are particularly susceptible to perturbations in mitochondrial function. Accordingly, many age related neurological diseases and other adverse conditions of the brain arising from drug effects or toxin exposure share common pathogenic processes that encompass mitochondrial dysfunction. Rotenone is a widely used pesticide and a potent inhibitor of mitochondrial complex I function. In this study, rotenone-induced effects on neuronal function were evaluated in mouse hippocampus; a primary brain region regulating memory formation. Can deleterious effects of rotenone be reversed or reduced by a synthetic lipoylcarnitine antioxidant compound; PMX-500F.

Methods. Rotenone (400 mg/kg/day) was administered orally to female ICR mice (3-4 mo of age) for one week. A separate group of mice were co-administered PMX-500F. Control mice were administered vehicle only (0.5% carboxymethyl cellulose solution). After one week of treatment, freshly prepared brain slices or whole hippocampi were isolated from anesthetized mice. Electrophysiological recordings were performed in live brain slices and the whole hippocampi homogenates were used for western blot analysis.

Results. Long term potentiation (LTP) was reduced by rotenone exposure. Potentiation during theta burst stimulation (TBS) was similar among the treatment groups. Neurotransmitter release, which underwent transient increase immediately after TBS, was lower in the rotenone treated mice. In rotenone treated mouse hippocampi these changes were accompanied by reduced basal synaptic transmission, increased BAX translocation to mitochondria, decreased BAD phosphorylation and decreased ERK 1/2 phosphorylation. Co-treatment with PMX-500F normalized these effects to control levels.

Conclusions. These results illustrate that an antioxidant can provide a protective effect against rotenone-induced impairment, in both excitatory synaptic physiology and activation of proapoptotic processes, in the mouse hippocampus.

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