

(KHS) maintained at 37°C, aerated with 95% O₂ and 5% CO₂. Following equilibration, viability of the valves was assessed by contraction with a potassium containing solution. Valves were then washed in KHS and all re-equilibrated. Following pre-contraction with norepinephrine (10⁻⁶ M), the cumulative relaxant effects of adenosine were assessed in parallel with a saline control. Cumulative relaxant effects of either the A₂ receptor selective agonist N-ethylcarboxamido adenosine (NECA; 10⁻¹¹ M to 10⁻⁷ M) or the A₁ receptor selective agonist cyclopentyladenosine (CPA; 10⁻⁹ M to 10⁻⁶ M) were assessed in paired valve segments. Relaxant responses were expressed as a percentage of the contractile force generated by norepinephrine. Cumulative response curves were fitted using non-linear regression. Data are presented as arithmetic mean ± sem (maximum response) or geometric mean with 95% CI (EC₅₀ values).

Adenosine caused significant relaxation of the equine aortic valve (P = 0.001) with a mean relaxation of 66% (±13.59). NECA was significantly more potent than CPA with EC₅₀ values of 14 X 10⁻⁹ (1.2 X 10⁻¹⁰ to 3.5 X 10⁻⁹) vs 13 X 10⁻⁷ (6.3 X 10⁻⁸ to 12.4 X 10⁻⁷) respectively (p < 0.01). The maximum relaxant responses to NECA and CPA did not differ significantly (87.7 ± 17.4 vs 103.6 ± 74.6) respectively.

These data show for the first time that valvular tissue possess receptors mediating relaxation in addition to those mediating contraction. Adenosine causes relaxation of the valve cusps through valvular A₁ receptors. The characterisation of adenosine mediated relaxation of the equine aortic valve contributes to our knowledge of valve function in the horse. Further studies are necessary to evaluate differences between normal and diseased valves and those with physiological regurgitation.

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ELECTROCARDIOGRAPHIC AND HEMODYNAMIC EFFECTS OF THE CALCIUM-CHANNEL BLOCKER DILTIAZEM IN HORSES. C.C. Schwarzwald, J.D. Bonagura, V. Luis Fuentes. Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.

Quinidine is an effective treatment for atrial fibrillation (AF) in horses, but often accelerates ventricular response rate. Diltiazem controls heart rate response to AF in other species, but has not been studied in horses. This investigation examined the effects of diltiazem on cardiac rate and rhythm, systolic and diastolic left ventricular (LV) function, central hemodynamics, and peripheral blood flow in normal, standing, non-sedated horses instrumented under local anesthesia.

Eight healthy horses were treated with IV diltiazem every 30 minutes to achieve cumulative dosages of 0 mg/kg (saline control), 1 mg/kg, 15 mg/kg, and 2 mg/kg. Doses were based on results of a previous dose-finding study. Plasma diltiazem concentration, ECG (HR, rhythm, PR interval), LV function (±dp/dt_{max}, LVEDP, *Tau*), central hemodynamics (RAP, PAP, aortic pressure, CO by thermodilution, SVR), LV dimensions (by echocardiography), and forelimb blood flow (by duplex Doppler sonography) were measured or calculated during each treatment period. Data were analyzed using one-way ANOVA for repeated measures. When mean values were statistically different (p < .05), Dunnett's test was used to compare treatment effects to baseline.

Diltiazem plasma concentrations between 390 and 910 ng/ml were achieved, with considerable variation among horses. Diltiazem increased mean ventricular rate insignificantly and increased atrial rate significantly. Variable degrees of sinoatrial and atrioventricular blocks were observed. The PR interval during conducted beats was prolonged significantly. Systemic blood pressure decreased, while right atrial, pulmonary arterial, and end-diastolic LV pressures increased significantly. Significant decreases in LV fractional shortening, ±dp/dt_{max} and -dp/dt_{max}, and a small but significant increase in *Tau* were measured. Cardiac output did not change, but stroke volume declined non-significantly (p = .506) at the highest dose range. Systemic vascular resistance decreased significantly at all treatment periods. Significant increase in diameter of the brachial artery and decrease in the resistive index of blood flow to the forelimb were demonstrated. Two horses developed high-grade sinus arrest with clinically significant systemic hypotension.

Cardiac effects of diltiazem, at 1 to 2 mg/kg IV, included mild impairment of systolic and diastolic LV function and intermittent depression of the sinus and AV nodes. Vascular effects of diltiazem included arterial vasodilation, increased limb blood flow, and decreased systemic vascular resistance. The fall in ABP seemingly invoked the baroreceptor reflex, causing sympathetic activation that increased sinus node rate, and presumably blunted the depressive effects of diltiazem on myocardial and nodal tissues. Diltiazem appears relatively safe in healthy horses, but dose is critical, and use may be limited by hypotension from vasodilation and direct suppression of sinus node discharge. Further studies are required to determine the pharmacokinetic profile of diltiazem, the potential frequency-dependence of diltiazem effects on nodal tissues, and the effects and safety of combined treatment with diltiazem and quinidine in horses with AF.

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DETERMINATION OF GLUTATHIONE AND LIPID PEROXIDATION IN FELINE PERIPHERAL BLOOD CELLS USING FLOW CYTOMETRY. C Webb, S Dow, M Lappin, A Guth, D Twedt. Colorado State University, Fort Collins, CO.

Reduced glutathione (GSH) plays a critical role in maintaining intracellular

oxidative balance and neutralizing potentially harmful free radicals. Cell membrane lipid peroxidation (LPO) is a common and deleterious consequence of oxidative imbalance and may result in loss of cell function, cell death, or hemolysis in the case of erythrocytes. There are very few clinically accessible assays for either of these important components of oxidative stress in samples from feline patients. This study investigates the use of flow cytometry to measure these parameters.

Sixty-nine clinical samples of feline peripheral blood were analyzed for GSH levels and susceptibility to lipid peroxidation. Monochlorobimane (mBCI) (Molecular Probes™) was used to determine relative intracellular GSH levels. mBCI conjugates with intracellular reduced glutathione to form a fluorescent product whose mean intensity is linearly correlated with GSH concentration. The fluorescence of the BODIPY581/591 (Molecular Probes™) fluorophore shifts from red to green upon peroxidation (stimulated by cumene hydroperoxide), allowing for the ratiometric measurement of lipid oxidation in live cells. The fluorescence of these molecular probe reactions is captured and analyzed using a Dako-Cytomation™ 3-laser 9-color Cyan flow cytometer.

The distinct scatter pattern and appropriate gating paradigm allowed for the separate analysis of neutrophils, monocytes, and lymphocytes from a sample in which the RBCs had been lysed (NH₄Cl). Cell identity was confirmed using fluorescent antibodies directed towards specific cell surface markers. The relative amount of GSH was significantly greater in neutrophils than in monocytes, and both cell types had significantly greater GSH levels than lymphocytes. The samples were then grouped according to diagnosis and included healthy controls, cats undergoing I¹³¹ treatment, anemic animals, cats with neoplasia, and 'other disorders'. The results suggest that diseased cats in general have significantly greater levels of intracellular leukocyte GSH than healthy animals.

Applying the BODIPY assay to 13 cats experimentally infected with hemobartonella (separate study) revealed that as the infection progressed two distinct populations of erythrocytes became apparent. Those RBCs with low levels of GSH had high levels of peroxidation, while those cells with high levels of GSH had lower levels of peroxidation.

Flow cytometry revealed distinct differences in important parameters of oxidative stress both between cell types (WBCs) and within a single cell type (RBCs). Furthering our understanding of the role of oxidative stress in feline diseases may help direct future intervention (i.e. GSH supplementation in cats with RBC parasitemia).

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LONG-TERM COMBINED ANTI-RETROVIRAL THERAPY (CART) IN FELINE IMMUNODEFICIENCY VIRUS INFECTED CATS: A CASE REPORT. J. Huebner¹, D. Klein², E. Müller, T.W. Vahlenkamp³, I. Langbein¹. ¹LABOKLIN, Bad Kissingen, Germany; ²Institute for Virology, University of Veterinary Medicine Vienna, Austria; ³Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

This case report describes the long-term treatment of a feline immunodeficiency virus (FIV)-infected cat with a combination of different anti-retroviral drugs. The cat showed clinical signs similar to the *AIDS Related Complex* (ARC) stage of the infection using the classification for human immunodeficiency virus (HIV) infection of the Center of Disease Control. (CDC). The combined anti-retroviral therapy (CART) consisted of the simultaneous application of ABC (ABACAVIR™), PMPA [9-(2-phosphonylmethoxyethyl)adenine] (VIREAD™), and 3TC (LAMIVUDIN™), drugs also used in highly active anti-retroviral therapy (HAART) treatment regimen in HIV-infected patients. The treatment was supplemented by feline Interferon-omega (IFNω).

Therapy was followed up by routine clinical investigations and by the measurement of several diagnostic parameters. Measurement of the immune status was performed using fluorescence activated cell sorter (FACS) analysis to determine the role of CART on the different lymphocytes subpopulations. The immune status was measured four times during a treatment period of one year. The results were compared with data obtained from 26 FIV-infected and 36 uninfected cats sent to our laboratory during this time period for routine diagnosis. In addition proviral load was determined from the cat receiving CART by real-time PCR.

During the treatment period the general health status of the cat improved, although the improvement was accompanied by intermittent periods of clinical disease. FACS analysis revealed that all T cell populations increased remarkably. The analysis of the CD4⁺/CD8⁺-cell ratio showed that CART resulted in a sustained increase with almost reference range values of normal uninfected cats at the end of the study. In concordance with the improvement of the immune status of the cat the proviral load decreased significantly by more than 2 log.

Previous studies using single drug treatment regimen in FIV-infected cats showed to be of only limited clinical value. To the best of our knowledge this is the first long-term follow up study of a diseased, chronically FIV-infected cat receiving CART. Data reveal that CART results in the improvement of the clinical and immunological status of infected cats and also results in reduced viral loads. Establishing of flow cytometric analysis in routine diagnostics is a new tool for therapy control of immune-mediated diseases, like FIV infection of cats. It should, however, be emphasized that CART requires a high degree of commitment to ensure the continuous application of the drugs.

