Electrocardiographic and Hemodynamic Effects of the Calcium-Channel Blocker Diltiazem in Horses

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ELECTROCARDIOGRAPHIC AND HEMODYNAMIC EFFECTS OF THE CALCIUM-CHANNEL BLOCKER DILTIAZEM IN HORSES. D. W. Schwarzwald, D.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 rate response. 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 (by duplex Doppler sonography) were measured or calculated during each treatment period. Data were analyzed using one-way ANOVA for repeated measures with the ratiometric measurement of lipid oxidation in live cells. The fluorescence intensity is linearly correlated with GSH concentration. 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 therapy, anemic animals, cats with neoplasia, and ‘other disorders’. The results suggest that diseased cats in general have significantly greater levels of intracellular leukocyte and platelet GSH than healthy animals.

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, if any, 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 (mBC) (Molecular Probes™) was used to determine relative intracellular GSH levels. mBC 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.

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 (ZIDOVUDINE™), 3TC (LAMIVUDINE™), EMTRASE (EMTRIDINE™), and ROGIVA (STI507™). After an improvement of the immune status of the cat the proviral load decreased significantly by more than 2 log.

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 ratio showed that CART was 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.

This study investigated the use of flow cytometry to measure these parameters. Measurement of the immune status was performed using fluorescence activated cell sorter (FACS) analysis to determine the role of CART on different lymphocyte 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.

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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 visible. Those RBCs associated with GSH high had levels of peroxidation, while those cells with high GSH had levels of lower peroxidation.

Flow cytometry revealed distinct differences in important parameters of oxidative stress both between cell types (RBCs) 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).
LONG-TERM COMBINED ANTI-RETROVIRAL THERAPY (CART) IN FELINE IMMUNODEFICIENCY VIRUS INFECTED CATS: A CASE REPORT

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abstract

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 combined anti-retroviral therapy (CART) was supplemented by feline Interferon-omega. Therapy was followed up by measurement of several diagnostic parameters. Measurement of the immune status using flow cytometry (FACS) was performed to determine the role of the anti-retroviral treatment on the different lymphocyte subpopulations. In addition proviral loads were determined by real-time PCR.

Introduction

The Feline Immunodeficiency Virus (FIV) is a member of the family of retroviruses, genan lentivirus. FIV has a tropism for T-cells and macrophages and causes a persistent lifelong infection characterised by four different clinical stages. After the infection, cats develop pyroxyria and a decrease of neutrophils in the blood. Usually, cats recover and become clinical asymptomatic. The stage as asymptomatic Carrier (AC) may last for months or years. A persistent, generalised lymphadenopathy is described as the third stage and is called AIDS Related Complex (ARC). Due to the progressive deterioration of immunological functions and a continued loss of CD4+ cells, cats develop clinical signs similar to the Acquired Immune Deficiency Syndrome (AIDS) described in HIV-infected patients. FIV has a world wide distribution with a variety of different strains and subtypes.

Material and methods

The FIV-infected cat

The described cat is a male, neutered domestic short hair and approximately 11 years old. He lived in Sri Lanka and was brought to Germany for medical treatment due to his poor and emaciated body condition. In January 2002 he was diagnosed with FIV. It is not known when the cat got infected with the virus, but several opportunistic bacterial and fungal infections causing serious problems over time argued against a more recent infection. Further investigations revealed renal failure, hypertrophic cardiomyopathy and diabetes mellitus. Haematological diagnosis showed a marked anemia predominantly caused by an infection with Haemobartonella (Mycoplasma haemofelis). Preliminary classification of a human immunodeficiency virus (HIV) infection of the Center of Disease Control (CDC) the cat was in the AIDS Related Complex (ARC) stage of the FIV-infection with a variety of different strains and subtypes.

Therapy and blood samples

Apart from continuous treatment with anti-microbial drugs, infections, cardiac and insulin therapy, an anti-viral treatment was started in November 2002. Following the highly active anti-retroviral therapy (HAART) treatment regimen in HIV-infected patients, the combined anti-retroviral therapy (CART) consisted of the simultaneous application of ABC (ABCavir), PMPA (PMPA (2- phosphonylmethoxythymidylicacid) (3TC) and ACC (ACAVID-β)). The use of AZT (Retrovir®) is not suitable for cats because of profound side effects including the development of anemia. AZT was therefore replaced with the nucleoside analogue PMPA. Whereas ABCavir and 3TC are available as liquid drug, PMPA is only available in tablets. Size and shape of the tablets are usually problematic. Therefore, the tablets were grinded to powder and mixed with water to produce small pills. The treatment was further supplemented by feline Interferon-omega (IFN-ω).

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Figure 1. Natural history of FIV infection

The drug regimen consisted of CART using 3TC, ABC, PMPA and sustained applications of feline Interferon-ω.

Figure 2: Immune status as determined by FACS analysis.

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 at the beginning of the study. This time period for routine diagnosis. FACS analysis revealed, that the B-cell numbers did not change during the observation period. Compared with the CD8+ cells at the first timepoint, both T-cellpopulations were lower in number at the second and third sample point. Over the entire treatment period, both CD4+ and CD8+ T-cells increased. Interestingly, the analysis of the CD4+/CD8+ cell ratios showed that CART resulted in a sustained remarkable increase with almost reference range values of normal uninfected cats at the end of the study.

measurement of proviral load

Isolation of DNA from 200µl EDTA-blood was performed with QIAamp Kit (Qiagen). Five µl of the isolated DNA were used for real-time PCR using primer FIV257F (5’-ATT GAC TCA G66 ACA ACA G66 AB-3’), FIV336F (5’-CCA G66 CTT CA66 CAT AT66 TAG AT-3’) and probe FIV281p (5’-FAM-CAC ACC AAG GTT TGC ACC AGC CAG GAT G-TAMRA-3’). 25 µl real-time PCR master mix included 50 µM of each primer, 200 nM of the probe, 3 mM of MgCl2, 200 µM of dATP, dCTP, dGTP and 400 µM of dUTP, 300X of both primer, 200 nM of the probe and 2 µl TOP Super PCR Polymerase (Promega). After denaturation for 2 min with 95 °C, 45 cycles with a profile of 10 sec with 95 °C and 1 min with 60 °C (ABI Prism 7700 SDS, Applied Biosystem) were performed.

The number of viral copies were calculated with Sequence Detection Software Version 1.7 (Applied Biosystem) using a cat specific standard in a logarithmic calibration with the reference value for cat lymphocytes. Results of the FACS analysis determining the percentage of CD4+ cells (CD4+) and CD8+ cells (CD8+) and the CD4/CD8 cell ratio (34) are shown below, that CART resulted in a sustained increase of the CD4+ cell ratio.

summary and conclusion

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 diagnosed, chronically FIV-infected cat receiving CART. Data reveal that CART results in the improvement of the clinical and immunological status of infected cats and overall results in reduced viral loads. Establishing of flow cytometric analysis in routine diagnostics is a new tool for therapy control of immunemediated diseases, like FIV infection of cats. It should, however, to be emphasised that CART requires a high degree of commitment to ensure the continuous application of the drugs.

Figure 3: Follow-up of proviral load. 

Figure 4: Domain status as determined by FACS analysis.