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Predictive value of natriuretic peptides in dogs with mitral valve disease

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Abstract

Natriuretic peptides are useful in diagnosing heart failure in dogs. However, their usefulness in detecting early stages of myxomatous mitral valve disease (MMVD) has been debated. This study evaluated N-terminal (NT) fragment pro-atrial natriuretic peptide (NT-proANP) and NT-pro-brain natriuretic peptide (NT-proBNP) in 39 Cavalier King Charles Spaniels (CKCS) with pre-clinical mitral valve regurgitation (MR), sixteen dogs with clinical signs of heart failure (HF) and thirteen healthy control dogs. Twenty seven CKCS and ten control dogs were re-examined 4 years after the initial examination and the status of the dogs 5 years after the initial examination was determined by telephone calls to the owner. All dogs were evaluated by clinical examination and echocardiography. CKCS with severe MR had higher NT-proANP and NT-proBNP compared to controls and CKCS with less severe MR. Dogs with clinical signs of HF had markedly elevated NT-proANP and NT-proBNP. Plasma concentrations of the natriuretic peptides measured at re-examination could predict progression in regurgitant jet size.

Keywords: NT-proANP; NT-proBNP; Mitral regurgitation; Heart failure; Cavalier King Charles Spaniels

Introduction

Atrial (ANP) and brain (BNP) natriuretic peptides are a family of structurally related peptides that function in the integrated control of renal and cardiovascular function (Levin et al., 1998). The natriuretic peptides decrease cardiac preload, suppress renin and aldosterone secretion and exert natriuretic functions. ANP and BNP are released by myocardial tissue primarily in response to increased stretch in the atrial and myocardial wall (van Wamel et al., 2000; Roncon-Albuquerque et al., 2006) and are increased in dogs with congestive heart failure (Asano et al., 1999; Haggstrom et al., 2000; MacDonald et al., 2003).

ProANP is stored intact in dense core granules in atrial cardiomyocytes. Increases in blood pressure or blood volume trigger the proteolytic cleavage of proANP to the mature C-terminal fragment (active hormone) and N-terminal (NT) fragment (NT-proANP), which are concomitantly released from secretory granules (Canaff et al., 1996). B-type natriuretic peptide, in contrast to ANP, is a constitutively secreted hormone with little intracellular storage. However, BNP gene expression increases substantially in response to volume overload (Mantymaa et al., 1993). The N-terminal fragments of ANP and BNP are released in equimolar concentrations to the mature C-ter-

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minal fragments (Levin et al., 1998). However, since the clearance is markedly lower than that of the mature C-terminal fragments, plasma concentrations of the N-terminal fragments are much higher than the mature hormone (Pemberton et al., 2000). Moreover, it has been found in humans that the biological variation of the N-terminal fragments is less than the mature C-terminal fragments and these may therefore be better suited for diagnostic purposes (McDowell et al., 2002; Fontana et al., 2007).

Natriuretic peptides have been shown to be useful in the diagnosis of heart failure in dogs with cough or dyspnoea (Prosek et al., 2007; DeFrancesco et al., 2007) and ANP and BNP appear to be potential predictors of survival in dogs with heart failure (Greco et al., 2003; MacDonald et al., 2003). Some studies have investigated the utility of natriuretic peptides in screening for occult or pre-clinical heart disease, with variable results (MacDonald et al., 2003; Oyama et al., 2007; DeFrancesco et al., 2007). Although some studies have evaluated NT-proANP as a marker for pre-clinical heart disease, most investigators have measured C-terminal BNP and no previous studies have investigated the usefulness of a canine specific NT-proBNP kit as a screening tool for occult heart disease or the ability to predict progression of disease.

The aims of the present study were to: 1) Compare plasma concentrations of NT-proANP and NT-proBNP in Cavalier King Charles Spaniels with varying degrees of myxomatous mitral valve disease (MMVD) prior to development of congestive heart failure (pre-clinical MMVD) to concentrations in healthy control dogs and dogs with clinical signs of heart failure; 2) To investigate the relation between natriuretic peptides and progression of MMVD.

Materials and methods

Dogs

Sixty eight privately owned dogs were studied. All dogs underwent clinical examination, echocardiography and blood sampling. Animals were excluded from the study if there was evidence of clinically relevant systemic disease other than heart disease, as determined by physical examination, haematology and serum biochemistry. Control dogs were excluded if there were any signs of heart disease on echocardiography. The study population was divided in three groups: Group 1 consisted of thirteen healthy control dogs, comprising two Golden Retrievers, two Labrador Retrievers, two Jack Russell Terriers, two mixed breed dogs and one each of a Dachshund, Chesapeake Bay Retriever, Bearded Collie, Kleiner Münsterländer and Cocker Spaniel. Group 2 consisted of 39 Cavalier King Charles Spaniels (CKCS) examined consecutively as part of a MMVD screening programme by the Danish Kennel Club. The CKCS had varying degrees of physical evidence of MMVD, but no clinical signs of heart failure. Group 3 included 16 dogs with MMVD and a clinical diagnosis of congestive heart failure, included as a positive control cohort. Eleven were CKCS and the remaining five dogs were small breed dogs with MMVD.

The clinical diagnosis of congestive heart failure was established by the presence of dyspnoea, combined with pulmonary oedema and/or venous congestion on thoracic radiographs, the latter which partially or completely resolved after 1–2 weeks of furosemide treatment. Group 1 and 2 dogs were examined at the Faculty of Life Sciences, University of Copenhagen, Denmark, from February 2002 to February 2003 and then re-examined from September to November 2006 at a median of 49 months later (range 36-57 months). Twelve CKCS and three control dogs were lost to follow-up. The status of all dogs in group 2 (death due to heart failure or due to other causes) was determined 12 months after the final examination by telephone calls to the owners. Group 3 dogs were examined at the Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences from October 2004 to May 2006.

Echocardiography

Echocardiography was performed under continuous electrocardiographic monitoring using a Vivid3 echocardiograph (GE-Medical). Dogs were unsedated. Right parasternal projections (short-axis) were used to measure heart dimensions. The left atrial and aortic root diameters were assessed in a short-axis 2-dimensional projection (Hansson et al., 2002). The ratio between these two measures (LA/Ao) was used as an index of left atrial size. The left ventricular end diastolic diameter (LVEDD) was assessed in short-axis M-mode at the level of the chordae tendinae (guided by a 2-dimensional short-axis view). Colour flow mapping of the mitral valve area was performed with a transducer at a Doppler transmitting frequency of 2.2 MHz using the left apical four chamber view of the dog in left lateral recumbency. Measurement of LA/Ao and LVEDD and assessment of mitral regurgitation (MR) by estimating the size (%) of the largest regurgitant jet occupying the left atrium was performed as previously described (Pedersen et al., 1999).

All echocardiographic examinations from dogs in groups 2 and 3 were recorded on videotape for later evaluation by observers who were blinded to the plasma levels of natriuretic peptides. Recordings from CKCS in group 2 examined in 2002 and 2006 were evaluated by the same investigator. Based on echocardiographic examinations, CKCS in group 2 were further divided into subgroups based on the degree of MR: dogs with no or minimal MR (regurgitant jet size $<20\%$), dogs with moderate MR (jet size $\geqslant 20\%$ and $<80\%$) and dogs with severe MR (jet size $\geqslant 80\%$).

Collection of blood

Blood was collected from the external jugular vein by venipuncture directly into two Vacuette tubes (Greiner) containing ethylenediamine tetraacetic acid (EDTA) for complete blood counts and measurement of plasma natriuretic peptide levels and one serum tube with clot activator for serum biochemistry. Plasma from one of the EDTA-treated blood samples was harvested by centrifugation at 3,000 *g* for 10 min at 5 °C and was processed within 30 min. Plasma was separated and all samples (from 2002 and 2006) were frozen and stored at −80 °C and batched for analysis in 2007 under similar conditions at the Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences. The serum tubes were kept at room temperature for a minimum of 20 min to allow clot formation before being centrifuged within 1 h of filling.

Assays for plasma NT-proANP and NT-proBNP

Plasma NT-proANP and NT-proBNP were analysed using commercial kits with antibodies raised against human NT-proANP1-98 (pro-ANP EIA, Biomedica Gruppe) and canine NT-proBNP (Canine Cardioscreen, VETSIGN, Guildhay) according to the manufacturers' instructions. All samples were assayed in duplicate. The detection limits were 50 pmol/L for the NT-proANP assay and 42 pmol/L for the NT-proBNP assay. Values below these limits were recorded as the lowest limit of detection. The intra-assay coefficients of variation (CV) were below 10% for the NT-proANP assay and below 15% for the NT-proBNP assay.

Statistical analyses

All statistical calculations were performed using statistical software (SAS statistical software, version 9.1, SAS Institute). Data were tested for normality by frequency distributions. Differences in age, body weight and echocardiographic variables were tested by Mann-Whitney and Student's *t*