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Prospective evaluation of Doppler echocardiography, tissue Doppler imaging and biomarkers measurement for the detection of doxorubicin-induced cardiotoxicity in dogs: A pilot study☆

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ABSTRACT

The purpose of this pilot study was to evaluate the usefulness of selected echocardiographic parameters, NT-proBNP and cardiac troponin I (cTnI) in the detection of cardiotoxicity in dogs treated with doxorubicin for various malignancies. Echocardiographic studies and biomarker measurements were performed before each administration of doxorubicin, then 1 and 3 months after completion of therapy. Thirteen dogs were included, with a total cumulative dose of doxorubicin ranging from 30 to 150 mg/m^2. E/A ratio significantly decreased during doxorubicin administration ($p = 0.047$). cTnI level was also significantly affected by treatment ($p = 0.046$), increasing above normal at least at one time point in 11 of 13 dogs.
The results of this pilot study suggest that monitoring of left ventricular diastolic function and cTnI level measurement might be useful in the early detection of cardiotoxic signs of doxorubicin therapy in dogs.

1. Introduction

Doxorubicin (DOX) is a potent chemotherapeutic agent, widely used in human and veterinary oncology. In dogs, this drug is part of the standard-of-care for treatment of a variety of cancers, including lymphoma, hemangiosarcoma and osteosarcoma (Chun et al., 2007). However, its use is limited by its myocardial toxicity, characterized by a delayed-onset cardiomyopathy that mimics dilated cardiomyopathy and carries a grave prognosis, through the development of refractory congestive heart failure or fatal arrhythmias (Van Vleet et al., 1980; Mauldin et al., 1992; Singal and Iliskovic, 1998). The reported incidence of cardiotoxicity in dogs treated with DOX is 8–64% depending on the chemotherapy protocol used and the criteria for diagnosis of toxicity (Mauldin et al., 1992; Sorenmo et al., 2004; Gillings et al., 2009; Ratterree et al., 2012). The occurrence of doxorubicin-induced cardiomyopathy (DC) is related to the cumulative dose of DOX administered (Von Hoff et al., 1979), leading to the recommendation in dogs that maximum lifetime dose should not exceed 180–240 mg/m^2 of body surface area (BSA) (Mauldin et al., 1992; Chun et al., 2007; Ratterree et al., 2012). Nonetheless, sensitivity to the drug varies among individuals, and cardiotoxic events have been observed at lower doses in dogs treated with DOX (Susaneck, 1983; Mauldin et al., 1992; Ratterree et al., 2012).

Guidelines for evaluation and follow-up of DOX-induced cardiotoxicity have not yet been defined in dogs and current screening methods lack sufficient predictive power (Ratterree et al., 2012; Tater et al., 2012). In people, serial endomyocardial biopsy has been historically considered the gold standard for diagnosis of anthracyclines related cardiotoxicity (Mason et al., 1978; Singal and Iliskovic, 1998). Non-invasive techniques are however preferred in practice, with ECG monitoring and echocardiographic follow-up of fractional shortening (FS) and left ventricular ejection fraction (LVEF) used traditionally (Mauldin et al., 1992; van Dalen et al., 2006; Galderisi et al., 2007). However, this approach is considered insensitive to detect subtle alterations in myocardial function; heart failure can occur despite a preserved systolic function and when changes are detected, cardiac dysfunction deteriorates rapidly and is irreversible (Barry et al., 2007;

Abbreviations: A_{max}, peak velocity of the late diastolic mitral wave; BNP, brain natriuretic peptide; BSA, body surface area; CHOP, cyclophosphamide-doxorubicin-vincristine-prednisone combination chemotherapy; cTn, cardiac troponin; DC, doxorubicin-induced cardiomyopathy; E/A, ratio of early to late diastolic left ventricular filling velocities; E', peak velocity of the pulsed wave TDI-derived early diastolic wave; EF, ejection fraction; E_{max}, peak velocity of the early diastolic mitral wave; EPSS, mitral valve E point to septal separation; ET, ejection time; FS, fractional shortening; IVCT′, TDI-derived isovolumic contraction time; IVRT′, TDI-derived isovolumic relaxation time; LA/Ao, left atrium to aorta ratio; LVIDd, left ventricle end-diastolic internal diameter; LVIDs, left ventricle end-systolic internal diameter; MPI, myocardial performance index; NT-proBNP, N-terminal proBNP; PEP, pre-ejection period; S′ peak velocity of the pulsed wave, TDI-derived systolic mitral wave; TDI, tissue Doppler imaging; UW-25, 25 weeks multidrug combination protocol from the University of Madison-Wisconsin; VPC, ventricular premature contraction; VT, ventricular tachycardia.

☆ This study was conducted at the Veterinary Teaching Hospital of the University of Montreal, Quebec, Canada.

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Jurcut et al., 2008). To improve early detection of toxic myocardial injury, new diagnostic tools have been evaluated in people. Assessment of conventional Doppler echocardiography has shown that changes in diastolic function are frequent and often precede systolic dysfunction in patients treated with DOX (Marchandise et al., 1989; Tassan-Mangina et al., 2006). In fact, early signs of cardiotoxicity are often related more to the filling pressures than to the systolic function (Barry et al., 2007). Evaluation of mitral inflow velocity profile by conventional Doppler echocardiography is used to characterize left ventricular diastolic function in people and dogs (Spirito et al., 1986; Nishimura et al., 1989), through measurement of peak velocities of early (E) and late (A) diastolic mitral waves, and determination of the ratio between these velocities (E/A ratio). In people, tissue Doppler echocardiography (TDI), a newer technique measuring myocardial velocities, appears sensitive in detecting early ventricular dysfunction secondary to DOX therapy (Kapusta et al., 2000; Tassan-Mangina et al., 2006; Nagy et al., 2008; Baysal et al., 2010). Myocardial performance index (MPI), a parameter of global left ventricular function, derived from conventional or tissue Doppler (Hori et al., 2007), has been reported to increase in patients treated with DOX (Santin et al., 2007; Dodos et al., 2008). These tools have not yet been evaluated in the context of canine DC, but early alterations in mitral inflow velocity profile, MPI, and TDI parameters, have been reported during the occult phase of dilated cardiomyopathy (Lee et al., 2002; Chetboul et al., 2004a, 2004b; O'Sullivan et al., 2007).

Cardiac biomarkers have been extensively studied in the last few years to assess their ability to improve diagnosis of DC in humans. Cardiac troponins (cTn) I and T are highly sensitive and specific markers of cardiomyocytes injury (Prosek and Ettinger, 2009). Some studies have suggested a relationship between cTn increase and myocardial dysfunction secondary to DOX therapy in people (Lipshultz et al., 1997; Cardinale et al., 2000; Mavinkurve-Groothuis et al., 2008), whereas others failed to show such association (Kismet et al., 2004; Koseoglu et al., 2005; Soker and Kervancioglu, 2005). In dogs, increase of cTn levels has been observed following administration of DOX (DeFrancesco et al., 2002; Selting et al., 2004a, 2004b). Dogs that developed clinical heart disease had an increase of cTnI that preceded recognition of cardiac dysfunction. Optimal timing for cTnI measurement after DOX administration remains controversial since ongoing cardiomyocyte loss continues after DOX administration, and therefore monitoring intervals are still exploratory (Selting et al., 2004a, 2004b).

B-type natriuretic peptide (BNP) is secreted by ventricles in response to increased workload. N-terminal proBNP (NT-proBNP) is an inactive fraction of BNP precursor, more stable in plasma than the active peptide (Prosek and Ettinger, 2009). Similarly to cTn, research in people has yielded conflicting results, but the ability of natriuretic peptides to predict DC has been observed in several studies (Soker and Kervancioglu, 2005; Dodos et al., 2008; Mavinkurve-Groothuis et al., 2008). In one study, an increase in BNP level was seen during DOX administration in 7 dogs, but of no clinical importance (Alves de Souza and Camacho, 2006). NT-proBNP has not been evaluated in this setting in dogs, but NT-proBNP measurement has proven useful in detecting occult DCM (Chetboul et al., 2004c; Oyama et al., 2007, 2008).

The primary objective of this study was to assess the effect of doxorubicin on cTnI, NT-proBNP levels, and selected Doppler and TDI parameters in dogs.

2. Materials and methods

2.1. Study population

All dogs presenting to the oncology department of the Veterinary Teaching Hospital of the University of Montreal between July 2008 and December 2009 to receive a doxorubicin-based chemotherapy treatment were considered for inclusion in the study. Written informed consent was obtained from owners before enrolment. Dogs were excluded if significant cardiac disease (i.e., precluding safe doxorubicin use or requiring specific cardiac treatment) was detected upon first cardiac evaluation, if DOX had already been administered before entering the study, or if the dog was lost to follow-up after initial evaluation and administration of doxorubicin. The study protocol was approved by the institutional Animal Care Committee.

2.2. Study design

Dogs were prospectively evaluated for cardiotoxicosis from the first administration of DOX until 3 months after the last administration. A complete cardiac evaluation including physical examination, 30 min ECG recording and complete echocardiography with TDI, was performed before each administration of the drug, then 1 month and 3 months after the last DOX treatment. Blood samples for measurement of cTnI and NT-proBNP were collected at the same time points.

2.3. Chemotherapy protocols

Dogs were treated with DOX either alone or in combination with other agents, as indicated by their medical condition, according to the protocol defined by a board-certified oncologist (MEN). Depending on the chemotherapy protocol, DOX was thus administered every 3–6 weeks.

After administration of diphenhydramine (1 mg/kg IM), doxorubicin was administered through an IV infusion over 20 min, as currently recommended at the authors' institution. The standard dose of 30 mg/m^2 BSA was used, unless specific dose adjustment was recommended by the oncologist, i.e. in small dogs (Arrington et al., 1994), or previous grade 3 or 4 adverse reaction to the drug (VCOG, 2004).

Doxorubicin administration was repeated to a maximal cumulative dose of 150 mg/m^2 BSA (5 sessions), depending on the chemotherapy protocol, the response to therapy and the occurrence of toxicities. However, in case of good response to chemotherapy and in the absence of adverse events, treatment could be pursued beyond 150 mg/m^2 BSA, if deemed beneficial to the dog by the oncologist and with the informed consent of the owner.

2.4. Cardiac evaluation

Cardiac auscultation, ECG recording and complete echocardiography were performed before each administration of DOX, then 1 month and 3 months after the last treatment. 6-lead computerized ECG[1] was recorded with the dogs in right lateral recumbency during a period of 5 min. Lead II monitoring was then maintained for arrhythmia detection during echocardiography. Heart rate and rhythm or conduction abnormalities were recorded. All echocardiographic examinations were performed by the same operator (JGL) on unsedated dogs, by use of an ultrasound system[2] equipped with a multi-frequency 4 MHz phased-array transducer. During echocardiography, animals were positioned in lateral recumbency while obtaining standard views (Thomas et al., 1993; Bélanger, 2009). The following echocardiographic parameters were specifically evaluated: fractional shortening (FS), mitral valve E point to septal separation (EPSS), bi-dimensional left atrium to aorta ratio (LA/Ao), left ventricular end-diastolic and end-systolic internal diameters (LVIDd and LVIDs, respectively) were obtained from the right parasternal short-axis view. Conventional Doppler and TDI measurements were obtained from the left apical 4-chamber view. Mitral inflow was recorded using pulsed-wave Doppler, with the sample volume positioned at the tip of the mitral leaflets and E/A ratio was calculated. Free wall mitral annulus motion was recorded using pulsed-wave TDI mode, with the filter set to exclude high-frequency signals and gains minimized to decrease background noise; the sample volume was placed at the level of the mitral annulus on the left ventricular free wall. Peak myocardial velocities during

[1] Marquette Mac 5000 Resting ECG, GE Medical System, Milwaukee, WI.
[2] Vivid 7 Vantage, GE System, Milwaukee, WI.