Serotonin Concentrations in Platelets, Plasma, Mitral Valve Leaflet, and Left Ventricular Myocardial Tissue in Dogs with Myxomatous Mitral Valve Disease

S.E. Cremer, G.E. Singletary, L.H. Olsen, K. Wallace, J. Häggström, I. Ljungvall, K. Höglund, C.A. Reynolds, N. Pizzinat, and M.A. Oyama

Hypothesis/Objectives: Altered serotonin (5-hydroxytryptamine, 5HT) signaling is postulated in development and progression of canine myxomatous mitral valve disease (MMVD). Little is known regarding platelet, plasma, valvular, or myocardial 5HT concentration ([5HT]) in affected dogs. We quantified [5HT] in platelet-rich plasma (PRP), platelet-poor plasma (PPP), mitral valve leaflets (MV), and left ventricular myocardium (LV).

Methods: High-performance liquid chromatography measured PRP, PPP, MV, and LV [5HT].

Results: Platelet-rich plasma platelet [5HT] was greater in CKCS CON (1.83 femtograms/platelet [fg/plt]; range, 0.20–4.76; P = .002), CKCS MMVD (1.58 fg/plt; range, 0.70–4.03; P = .005), and non-CKCS MMVD (1.72 fg/plt; range, 0.85–4.44; P = .003) versus non-CKCS CON (0.92 fg/plt; range, 0.63–1.30). There was no group difference in PPP [5HT]. MV [5HT] was significantly higher in MMVD (32.4 ng/mg; range, 8.4–106.7) versus non-HD (3.6 ng/mg; range, 0–28.3; P = .01) and LV [5HT] was significantly higher in MMVD (411.9 ng/mg; range, 4.0–104.8) versus other-HD (0.9 ng/mg; range, 0.6–9.9; P = .001).

Conclusions and Clinical Importance: Platelet [5HT] was highest in healthy CKCS and both MMVD groups, but plasma [5HT] showed no group differences. Tissue [5HT] was highest in MV and LV of MMVD-affected dogs, suggesting altered 5HT signaling as a potential feature of MMVD. Interactions of platelet, valvular, and myocardial 5HT signaling warrant further investigation.

Key words: 5-Hydroxytryptamine; Cavalier King Charles Spaniels; Heart disease; Myxomatous mitral valve disease.

Serotonin (5-hydroxytryptamine, 5HT) is involved in many aspects of valvular and myocardial function, in embryogenesis as well as in disease. During embryogenesis, 5HT, its receptors (R) and the serotonin reuptake transporter are needed for normal embryologic development of the cardiac valves, and the 5HT system likely plays a role in the homeostasis of normal valvular and myocardial function. An association between markedly increased plasma 5HT concentrations and valvular degeneration is well established in human patients suffering from 5HT-producing carcinoid tumors leading to a high prevalence of acquired valvular disease. Exogenous 5HT administration to

From the Department of Veterinary Disease Biology, University of Copenhagen, Frederiksberg, Denmark (Cremer, Olsen); the Departments of Clinical Studies (Singletary, Reynolds, Oyama); the Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA (Wallace); the Department of Clinical Sciences, (Häggström, Ljungvall); the Department of Anatomy, Physiology and Biochemistry, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Uppsala, Sweden (Höglund); and the Institut National de la Santé et de la Recherche Médicale (INSERM), Institut de Médecine Moléculaire de Rangueil, Toulouse, France (Pizzinat).

Corresponding author: Dr M.A. Oyama, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104; e-mail: maoyama@vet.upenn.edu.

Submitted February 22, 2014; Revised May 8, 2014; Accepted June 19, 2014.

Copyright © 2014 by the American College of Veterinary Internal Medicine.

DOI: 10.1111/jvim.12420

Abbreviations:

SHT 5-hydroxytryptamine
αSMA alpha-smooth muscle actin
ARVC arrhythmogenic right ventricular cardiomyopathy
CKCS Cavalier King Charles Spaniels
dCM dilated cardiomyopathy
ECM extracellular matrix
EDTA ethylene diamine tetra acetic acid
HPLC high-performance liquid chromatography
iAo indexed aortic diameter
iLAD indexed left atrial diameter
iLVIDd indexed end-diastolic left ventricular internal dimension
iLVIDs indexed end-systolic left ventricular internal dimension
ISACHC international small animal cardiac health council
LA : Ao left atrial to aortic diameter ratio
LV left ventricle
MMVD myxomatous mitral valve disease
MR mitral regurgitation
MV A peak velocity of the mitral inflow A wave
MV E peak velocity of the mitral inflow E wave
MV mitral valve
PPP platelet-poor plasma
PRP platelet-rich plasma
SERT serotonin reuptake transporter
TGFβ transforming growth factor beta
TPH1 tryptophan hydroxylase 1
VIC valvular interstitial cell
rats also has been linked to valvular heart disease.\textsuperscript{7,8} Furthermore, platelet-derived 5HT caused activation of myocardial fibroblasts in vitro, an indication that myocardial tissue also can be affected by platelet 5HT.\textsuperscript{9} Several lines of evidence support a role for 5HT in the pathogenesis of canine myxomatous mitral valve disease (MMVD).\textsuperscript{4,8,10–15} The degenerative changes of MMVD are characterized by an overproduction and deposition of extracellular matrix with disruption of collagen content and organization in the leaflet and chordae tendineae.\textsuperscript{16,17} Remodeling within the mitral valve (MV) is mediated by activation of normally quiescent valvular interstitial cells (VIC), by both mechanical and chemical mechanisms that are not fully understood.\textsuperscript{18,19} Serotonin has been linked to VIC activation in several species, including humans, rats, and dogs,\textsuperscript{20–23} and several studies have shown altered local 5HT signaling in canine myxomatous mitral valves.\textsuperscript{14,15,19,20,23–26}

Potential sources of increased 5HT signaling in dogs with MMVD include (1) increased local valvular production of 5HT, as suggested by increased valvular tryptophan hydroxylase 1 (TPH1), the rate limiting enzyme in 5HT production, in early- and late-stage MMVD\textsuperscript{24} and (2) increased platelet-derived serum 5HT concentration, primarily in dogs with early stages of MMVD.\textsuperscript{27–29} Serum 5HT in healthy Cavalier King Charles Spaniels (CKCS) is higher than serum 5HT concentrations in healthy dogs of other breeds, which could help explain the high, and also age-dependent, prevalence of MMVD in the CKCS.\textsuperscript{28}

Platelet, plasma, or locally produced 5HT may affect myocardial and MV tissue. Virtually all circulating 5HT is contained within platelets, and it is released into serum only during platelet aggregation and activation. Platelet-derived 5HT is directly involved in the activation of myocardial fibroblasts, and in the expression of alpha-smooth muscle actin, transforming growth factor beta (TGFβ), and matrix metalloproteinases, and this response is mediated, at least in part, by the 5HT\textsubscript{2A}R.\textsuperscript{9} Increased myocardial fibrosis and arterial changes have been reported in dogs with congestive heart failure (CHF) because of MMVD.\textsuperscript{30} Despite this previous data, little is known specifically about valvular, myocardial, plasma, or platelet 5HT concentrations in MMVD-affected dogs. In the current study, we hypothesized that higher platelet, plasma, MV leaflet, and left ventricular (LV) myocardial 5HT concentrations would be present in MMVD-affected dogs versus control dogs and dogs with non-MMVD heart disease.

**Materials and Methods**

The study was divided into plasma and tissue studies and performed in collaboration among the University of Pennsylvania (PENN), the University of Copenhagen (CPH), and the Swedish University of Agricultural Sciences (SLU). The studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committee, the Danish Inspectorate for Animal Experimentation, and the Local Ethical Committee in Uppsala, Sweden, and written owner consent was obtained for all animals.

**Plasma Study**

**Dogs.** Dogs >3 years of age were prospectively recruited among local breeders or clients associated with PENN and CPH from fall 2010 to summer 2011. Exclusion criteria included thrombocytopenia (platelet count <100,000/µL), macrothrombocytes, and presence of other systemic disease. Dogs with cardiac disease other than MMVD (eg, congenital heart disease, pericardial effusion) also were excluded from the study. All dogs were assessed by physical examination, echocardiography, electrocardiography, CBC, and serum biochemical profile. The dogs were separated by breed into CKCS and non-CKCS groups and by disease status into those with and without (CON) presence of MMVD. Myxomatous mitral valve disease was diagnosed based on presence of a left apical systolic murmur and echocardiographic evidence of color flow mitral regurgitation (MR) or mitral leaflet thickening and prolapse or both. The 4 groups were CKCS CON, non-CKCS CON, CKCS MMVD, and non-CKCS MMVD.

**Echocardiography.** Echocardiographic examinations\textsuperscript{31} were performed by cardiologists or trainees under the direct supervision of a cardiologist, using an echocardiographic unit\textsuperscript{a} with 3S and 5S transducers or a unit\textsuperscript{b} with s8-3 or s5-1 transducers. Modalities recorded included M-mode, 2D, and color flow Doppler. Presence of MR was assessed from right parasternal long-axis view, or left apical 4-chamber view or both. Two-dimensional measurements of the left atrial aortic root diameters from the right short axis view\textsuperscript{32} were averaged across 3 heart cycles. Either 2D or M-mode right parasternal short axis views were used to average end-diastolic and end-systolic internal left ventricular diameter across 3 cardiac cycles. Mitral inflow velocities were measured using pulsed wave Doppler from the left apical view. Measurements were performed by 1 of 2 observers (MAO and LHO) using off-line image processing and measurement software.\textsuperscript{c} Diastolic left atrial (LAD) and aortic (Ao) diameter and systolic and diastolic left ventricular diameters (LVIDd and LVIDs, respectively) were indexed to body weight according to previously published formulas\textsuperscript{33}: iLAD = LAD/body weight (BW)\textsuperscript{0.435}, iAo = LVIDs/BW\textsuperscript{0.414}, iLVIDd = LVIDd/BW\textsuperscript{0.315}, and iLVIDs = LVIDs/BW\textsuperscript{0.264}.

**Blood Sampling and Preparation of Platelet-Rich Plasma and Platelet-Poor Plasma.** Up to 15 mL of blood was collected from the jugular (CPH) or a peripheral vein (PENN) into EDTA tubes for CBC, sodium citrate (3.8%) tubes for preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) for 5HT high-performance liquid chromatography (HPLC) analyses and automated platelet count, and serum tubes for biochemical profiles. After blood collection, citrate tubes were incubated at room temperature for 15 minutes before centrifugation at 1,200 x g for 3 minutes. The supernatant PRP was collected and stored at −80°C. Remaining plasma was centrifuged at 3,000 x g for 10 minutes at room temperature, the supernatant PPP was collected and stored at −80°C. Platelet counts were performed on EDTA whole blood, PRP, and PPP on hematology analyzers.\textsuperscript{d}

**Tissue Study**

**Dogs.** Dogs associated with PENN, CPH, and SLU were recruited upon time of elective euthanasia. Owner consent to collect cardiac tissue was obtained and dogs were categorized into 3 groups. Two groups were euthanized because of end-stage heart disease because of either MMVD (MMVD) or non-MMVD car-
diac disease (other-HD). The control group (non-HD) included dogs without cardiac disease euthanized for noncardiac causes. Diagnoses were based on medical history including any chronic treatment for CHF, results of previous echocardiographic examination if available, and gross inspection of the heart and lungs at necropsy.

Procedures. The heart was collected postmortem and examined within 30 minutes of euthanasia. The entire anterior MV leaflet including associated chordae tendineae was collected. A 1 cm³ LV myocardial sample was collected from the atrioventricular groove, immediately below the level of the anterior descending coronary artery. Tissue samples were snap-frozen in liquid nitrogen and stored at −80°C.

High-Performance Liquid Chromatography. All PRP, PPP, and tissue samples were shipped on dry ice for HPLC analysis performed by 1 investigator (NP) as previously described. B Briefly, 5HT was extracted from PRP and PPP with Chromsystems reagent using an Internal Standard. Extracted 5HT was quantified electrochemically at 0.65 V and concentrations were calculated in nanograms per milliliter. Concentration of 5HT was further normalized to platelet count in PRP to estimate platelet 5HT concentration. MV or LV were weighed and extracted in 5 volumes (vol/wt) of 0.1 N perchloric acid/0.05% disodium EDTA/0.05% sodium metabisulfite. Extracted samples were injected onto a Beckman Ultrasphere 5-μm IP column and 5HT concentrations were calculated in nanograms per milligram.

Statistical Methods

Summary statistics describing the experimental groups was tabulated and are reported as median and range unless otherwise indicated. Statistical calculations were performed by statistical software. Overall differences among groups were analyzed using Fisher's exact test. Associations of 5HT concentration with age, weight, ISACHC class, LA/Ao, iLVId, and iLVIDs were determined by calculating Spearman rank correlation coefficients. Significance was defined as P < .05.

Results

Plasma Study

Dogs. Sixty dogs were recruited. Eight dogs were excluded because of thrombocytopenia or presence of macrothrombocytes and 7 dogs were excluded because of the automated cell reader's inability to obtain accurate EDTA or PRP platelet counts. Thus, 45 dogs were used for analysis (CPH, 25/45, 55.6%; PENN, 20/45, 44.4%). The following breeds were represented: CKCS (n = 26), mixed breed (n = 2), Dachshund (n = 2), Shih Tzu (n = 2), Short-haired Pointer (n = 2), and 1 each from the following breeds: Boston Terrier, Pug, Corgi, Norfolk Terrier, Sheltie, West Highland White Terrier, Pomeranian, Dalmatian, Poodle, Chihuahua, and Maltese. Group characteristics of age, weight, sex, and echocardiographic measurements are listed in Table 1.

Platelet Counts. The median EDTA platelet count of all 45 plasma study dogs was 312,000/μL (range, 133,000–693,000/μL). Significantly, more platelets were present in PRP versus PPP (PRP, 389,000/μL; PPP, 7,000/μL; P < .0001). There was no difference in median EDTA, PRP, or PPP platelet count across groups (Table 1).

PRP and PPP 5HT Concentrations. The overall median 5HT concentration was higher in PRP (620 ng/mL; range, 50–1380) than PPP (1.76 ng/mL; range, 0–394, P < .0001) consistent with the fact that

Table 1. Group summary (plasma study).

|        | Group 1 (N = 8) | Group 2 (N = 12) | Group 3 (N = 14) | Group 4 (N = 11) |
|--------|----------------|------------------|------------------|------------------|
| Age (years) | N = 45 | 4.5 (3–8) | 4.5 (3–6) | 10.5 (4–15) | 9.0 (7–14) |
| Weight (kg) | N = 45 | 14.6 (7.0–41.7) | 8.1 (6.6–13.1) | 9.8 (6.9–19.9) | 7.2 (2.9–25.9) |
| Sex (female/male) | N = 45 | 6/2 | 6/6 | 9/5 | 5/6 |
| Murmur (0/1/2/3/4/5/6) | N = 45 | 8/0/0/0/0/0/0 | 12/0/0/0/0/0/0 | 0/1/3/5/2/3/0 | 0/0/0/2/7/2/0 |
| ISACHC class (0/1a/1b/2/3a) | N = 45 | 8/0/0/0/0 | 12/0/0/0/0 | 0/2/8/2/2 | 0/3/6/2 |
| iLVId (mm) | N = 45 | 1.51 (1.17–1.66) | 1.37 (1.23–1.65) | 1.90 (1.41–2.63) | 1.98 (1.61–2.38) |
| iLVIDs (mm) | N = 45 | 0.99 (0.97–1.15) | 0.94 (0.72–1.21) | 1.1 (0.86–1.78) | 1.03 (0.70–1.36) |
| iLAD (mm) | N = 45 | 0.75 (0.59–0.92) | 1.00 (0.77–1.22) | 1.33 (0.94–2.11) | 1.40 (1.21–2.01) |
| iAoD (mm) | N = 45 | 0.75 (0.59–0.92) | 0.74 (0.46–0.91) | 0.76 (0.62–0.86) | 0.70 (0.57–0.84) |
| LA : Ao (ratio) | N = 45 | 1.30 (1.23–1.81) | 1.32 (1.14–1.47) | 1.83 (1.11–2.82) | 2.07 (1.60–3.12) |
| MV E (m/s) | N = 27 | 0.61 (0.44–0.70) | 0.65 (0.61–0.83) | 0.74 (0.49–1.38) | 1.23 (1.04–1.66) |
| MV A (m/s) | N = 25 | 0.45 (0.34–0.66) | 0.52 (0.45–0.78) | 0.67 (0.58–0.97) | 0.75 (0.34–1.20) |
| Ph EDTA (μL) | N = 45 | 333.5 (218–693) | 292.5 (156–522) | 326.5 (133–439) | 396 (237–529) |
| Ph PRP (μL) | N = 44 | 440 (159–1191) | 363 (112–551) | 486.5 (134–613) | 342 (126–530) |
| Ph PPP (μL) | N = 44 | 6.5 (2–12) | 11 (2–38) | 7 (3–43) | 7 (3–54) |

The table lists age, weight, sex, murmur, ISACHC class, echocardiographic data including, indexed end-diastolic left ventricular internal dimension (iLVIDd), indexed end-systolic left ventricular internal dimension (iLVIDs), indexed aortic diameter (iAoD), indexed left atrial diameter (iLAD), the ratio of left atrial to aortic root ratio (LA/Ao), peak velocity of the mitral inflow E wave (MV E), peak velocity of the mitral inflow A wave (MV A) and platelet counts (Ph) in EDTA white blood (EDTA), platelet-rich plasma (PRP) and platelet-poor plasma (PPP) in Cavalier King Charles Spaniels (CKCS) and non-CKCS without (CON) and with myxomatous mitral valve disease (MMVD); CKCS CON, Non-CKCS CON, CKCS MMVD and non-CKCS MMVD. Within each row, superscript numerals indicate that the group is statistically significant different from CKCS CON1, non-CKCS CON2, CKCS MMVD3, and non-CKCS MMVD4. Superscripts that are not italicized indicate that the respective P is <.05 and italicized superscripts indicate that the respective P-value is <.01.
Platelets are the main source of circulating 5HT. Median PRP platelet 5HT content was 1.62 femtograms/platelet (fg/plt) with a range of 0.20–4.76 fg/plt and differed between the groups (P = .003) (Fig 1). Median PRP platelet 5HT concentrations were higher in CKCS CON (1.83 fg/plt; range, 0.20–4.76; P = .002), CKCS MMVD (1.58 fg/plt; range, 0.70–4.03; P = .005), and non-CKCS MMVD (1.72 fg/plt; range, 0.85–4.44; P = .003) compared to non-CKCS CON (0.92 fg/plt; range, 0.63–1.30). The median PRP platelet 5HT concentration was not different between CKCS CON and CKCS MMVD (P = .19), CKCS CON and non-CKCS MMVD (P = .32), or CKCS MMVD and non-CKCS MMVD (P = .51). PRP platelet 5HT concentration was not significantly correlated with patient age (rho = 0.027, P = .86), weight (rho = −0.208, P = .17), ISACHC class (χ² = 0.785, P = .27), LA : Ao (rho = 0.042, P = .78), iLVIDd (rho = 0.033, P = .83), or iLVIDs (rho = 0.10, P = .51).

There was no difference in median PPP 5HT concentration among the groups (P = .18; data not shown).

Tissue Study

Dogs. Twenty-four dogs were included in the study (PENN, 14/24, 58.3%; CPH, 5/24, 20.8%; SLU, 5/24, 20.8%), the following breeds were represented: MMVD group, CKCS (n = 4), and 1 Jack Russell Terrier, Chihuahua, Cocker Spaniel, and Toy Poodle; other-HD group, Doberman (n = 3), mixed breed (n = 2), and 1 Boxer and Great Dane each; non-HD group, Beagle (n = 3) and 1 mixed breed, Bassett hound, Cane Corso, Welsh Terrier, CKCS, and German Shepherd. Cause of euthanasia in the other-HD group included dilated cardiomyopathy (DCM; n = 6) and arrhythmogenic right ventricular cardiomyopathy (ARVC; n = 1). Cause of euthanasia in the non-HD group included hemoabdomen (n = 2), hip dysplasia (n = 1), epilepsy (n = 1), and age-associated debilitation (n = 1). The remaining 3 dogs in this group were healthy purpose-bred Beagles that were euthanized as part of an unrelated study. There was no difference in sex among groups (P = .44) with 3 females and 5 males in the MMVD group, 2 females and 5 males in the other-HD group, and 5 females and 4 males in the non-HD group. The groups differed in age (P = .003) with a median age of 10.5 years (range, 8–16) in the MMVD group, 6 years (range, 3–10) in other-HD, and 5 years (range, 0.8–10) in non-HD.

Mitral Valve and Left Ventricular 5HT concentrations. Because of technical difficulties, HPLC of mitral valve tissue from 5 dogs could not be performed. Median values of both MV and LV 5HT concentration differed among groups (MV, P = .033; LV, P = .0024). Median MV 5HT concentrations of the MMVD group (n = 8; 32.4 ng/mg; range, 8.4–106.7) were higher than the non-HD group (n = 6; 3.6 ng/mg; range, 0–28.3;
the concentrations in the other-HD group (n = 5; 2.4 ng/mg; range, 0–71.7; P = .11; Fig 2A). Median MV 5HT concentrations were not different between the other-HD and non-HD group (P = .78). MV 5HT concentration was not correlated with sex (z = −1.44, P = .15) or age (r = 0.33, P = .16).

Median LV 5HT concentrations of the MMVD group (11.9 ng/mg; range, 4.0–104.8) were greater than the concentrations in the other-HD (0.9 ng/mg; range, 0–10.1; P = .011) and non-HD (2.5 ng/mg; range, 0–6.9; P = .001) groups (Fig 2B). LV 5HT concentrations were not different between the other-HD and non-HD groups (P = .96). LV 5HT concentration was not correlated with sex (z = 0.77, P = .44) or age, rho (r = 0.26, P = .24).

**Discussion**

To the authors’ knowledge, this is the first report of increased 5HT concentrations in platelets, mitral valve leaflets, and left ventricular myocardium in dogs with naturally occurring MMVD. The finding of increased platelet 5HT concentration in healthy CKCS and MMVD-affected dogs versus healthy non-CKCS dogs agrees with previous studies of 5HT concentration in serum samples and demonstrates that these studies measured platelet 5HT released upon clot formation, as expected. The current finding that plasma 5HT was not significantly different among groups does not support plasma 5HT in the pathogenesis of MMVD. However, stratification of MMVD severity could reveal a different picture.

The range of platelet 5HT content in healthy humans has been reported as 0.51–0.95 fg/plt with a maximum capacity of 3.5–7.9 fg/plt. Thus, in the current study, the platelet content in healthy non-CKCS dogs (0.92 fg/plt) is in agreement with previous data, and the 5HT platelet content in healthy CKCS or dogs with MMVD represents a 1.7- to 2-fold increase in normal platelet 5HT content found in humans. The increases in serum or platelet 5HT concentrations detected in predisposed and affected dogs generally agree with concentrations found in humans with carcinoid syndrome. Serotonin secretion is variable in presence of carcinoid tumors, but as many as two-thirds of patients have platelet 5HT content >0.95 fg/plt. In a study of human patients with chronic CHF, platelet 5HT concentration was increased 3.5 to 18-fold over controls, suggesting that increased platelet 5HT could be an epiphenomenon of the heart failure phenotype. The study cohort, however, included a mixture of valvular, ischemic, and hypertensive heart disease. In the dog, the current results, as well as those reported in previous studies, identified increased platelet or serum 5HT concentrations in healthy CKCS, indicating that increased 5HT is not solely a result of the heart failure phenotype. Cavalier King Charles Spaniels are highly predisposed to MMVD and considering the current and previous findings of increased platelet or serum 5HT in CKCS without clinical signs of MMVD, it is tempting to hypothesize involvement of platelet 5HT in the development and progression of MMVD in this breed. In a previous study, serum 5HT concentration decreased with increasing MMVD severity, suggesting a potential role of platelet 5HT in early stages of MMVD. In the present study, platelet 5HT concentration was not significantly associated with ISACHC or echocardiographic heart size. This observation could be because of the relatively low number of patients included with advanced MMVD.

Mitrval valve 5HT concentration from dogs with MMVD was 9-fold greater than in dogs with noncardiac disease, and 13.5-fold greater than in DCM/ARVC dogs. The latter was not statistically significant, likely because of the small group sizes and the large variation in 5HT concentrations. The source of increased MV 5HT in the present study is unknown but likely involves local 5HT production. The present study revealed significantly higher LV 5HT concentrations in MMVD dogs versus both noncardiac conditions and dogs with DCMA/ARVC, which suggests either local 5HT production within the myocardial tissue or exposure to platelet-derived 5HT. Regardless of the source of 5HT, the current report supports altered 5HT signaling as a specific feature of MMVD and subsequent LV remodeling.

The relationship between platelet, MV, and LV 5HT content remains unclear and the relative roles of platelet versus tissue-derived 5HT in the pathogenesis of MMVD and left ventricular remodeling merits further study. The increased platelet or serum 5HT concentration in MMVD-predisposed healthy CKCS suggests that platelet-derived 5HT may contribute to early stages of MMVD or LV remodeling. After myocardial injury, platelet activation is among the first responses. Platelet lysate, and in particular platelet-derived 5HT, induces activation, migration, and proliferation of cardiac fibroblasts in vitro, all of which are important steps in tissue remodeling. After injury, antagonism of the 5HT2AR significantly decreases infarct size. Previous studies indicated platelet dysfunction in CKCS and other dogs breeds with MMVD, but the exact nature and role of these abnormalities is controversial. Although this study did not investigate the source of platelet or tissue 5HT, local cardiac tissue production of 5HT is supported by the previously reported finding of increased TPH1, 5HT2B-R, and TGFβ1 in mildly MMVD-affected valves.

There are important limitations to the current study. The design is observational and is limited by the relatively small numbers of dogs included. Data regarding concurrent cardiac medications were inconsistently available and drug administration could have affected the results. Analyses of platelet function were not performed and dogs with macrothrombocytopenia were not included. The method to generate PRP did not enrich samples to the extent reported in previous studies, but this was accounted for by adjusting 5HT concentration to PRP platelet count. Paired PPP samples had low platelet counts and low overall 5HT concentrations indicating that the source of 5HT measured in the
Platelet, Valvular, and Myocardial 5HT Concentrations in MMVD

Platelets. Myocardial remodeling is a complex process and LV samples were limited to a single site and were obtained without concurrent histologic or additional 5HT pathway component examination.

In conclusion, platelet 5HT content was significantly higher in dogs with MMVD versus healthy non-CKCS dogs. Moreover, platelet 5HT content was higher in healthy CKCS compared to healthy dogs of other breeds, supporting potential involvement of platelet-derived 5HT in the pathogenesis of MMVD in this breed. Plasma 5HT was not significantly different among groups and does not support involvement of plasma 5HT in the pathogenesis of MMVD. MV and LV 5HT concentration were increased in MMVD-affected dogs indicating that altered tissue 5HT signaling is an important feature of MMVD. The role of platelet, valvular, and myocardial 5HT signaling in the pathogenesis of MMVD warrants further investigation.

Footnotes

a Vivid i ultrasound system; GE Healthcare, Broomfield, CO
b Philips Healthcare iE33 ultrasound machine, Andover, MA
c EchoPAC PC Version 112; GE Healthcare or Xcelera system online measurement
d Ca530 Vet, Boule Nordic AB; Kastrup Denmark; Cell-Dyn 3500, Abbott, Genofte, Denmark; scil Animal Care; Garnee, IL
b Chromsystems Instruments & Chemicals, Gräfelfing, Germany
f ESA. Coulouched III; Eurosep instruments, Cergy, France
g Beckman, Gagny, France
h Stata v12; Stata Corporation, College Station, TX

Acknowledgments

The authors thank Jo Anne Winget, Carolyn Michel, and Christina Tirsdal Kjempff for their technical assistance. The study was funded by the American Kennel Club-Canine Health Foundation. Conflict of Interest Declaration: The authors disclose no conflict of interest.

References

1. Nebigil CG, Maroteaux L. A novel role for serotonin in heart. Trends Cardiovasc Med 2001;11:329–335.
2. Pavone LM, Norris RA. Distinct signaling pathways activated by “extracellular” and “intracellular” serotonin in heart valve development and disease. Cell Biochem Biophys 2013;67:819–828.
3. Pavone LM, Spina A, Rea S, et al. Serotonin transporter gene deficiency is associated with sudden death of newborn mice through activation of TGF-beta1 signaling. J Mol Cell Cardiol 2009;47:691–697.
4. Mekontso-Dessap A, Brouri F, Pascal O, et al. Deficiency of the 5-hydroxytryptamine transporter gene leads to cardiac fibrosis and valvulopathy in mice. Circulation 2006;113:81–89.
5. Buskohl PR, Sun ML, Thompson RP, Butcher JT. Serotonin potentiates transforming growth factor-beta3 induced biomechanical remodeling in avian embryonic atrioventricular valves. PLoS One 2012;7:e42527.
6. Fox DJ, Khattar RS. Carcinoid heart disease: Presentation, diagnosis, and management. Heart 2004;90:1224–1228.
7. Rothman RB, Baumann MH. Serotonergic drugs and valvular heart disease. Expert Opin Drug Saf 2009;8:317–329.
8. Droogmans S, Roosens B, Cosyns B, et al. Dose dependency and reversibility of serotonin-induced valvular heart disease in rats. Cardiovasc Toxicol 2009;9:134–141.
9. Yabanoglu S, Akkiki M, Seguelas M, et al. Platelet derived serotonin drives the activation of rat cardiac fibroblasts by 5-HT2A receptors. J Mol Cell Cardiol 2009;46:518–525.
10. Xu J, Jian B, Chu R, et al. Serotonin mechanisms in heart valve disease II: The 5-HT2 receptor and its signaling pathway in aortic valve interstitial cells. Am J Pathol 2002;161:2209–2218.
11. Elangbam CS, Job LE, Zadrozny LM, et al. 5-hydroxytryptamine (5HT)-induced valvulopathy: Compositional valvular alterations are associated with 5HT2B receptor and 5HT transporter transcript changes in Sprague-Dawley rats. Exp Toxicol Pathol 2008;60:253–262.
12. Gustafsson B, Tommeras K, Nordrum I, et al. Long-term serotonin administration induces heart valve disease in rats. Circulation 2005;111:1517–1522.
13. Hutcheson JD, Ryzhoiva LM, Setola V, Merryman WD. 5-HT(2B) antagonism arrests non-canonical TGF-beta1-induced valvular myofibroblast differentiation. J Mol Cell Cardiol 2012;53:707–714.
14. Oyama MA, Levy RJ. Insights into serotonin signaling mechanisms associated with canine degenerative mitral valve disease. J Vet Intern Med 2010;24:27–36.
15. Orton EC, Lacfera CM, MacLea HB. Signaling pathways in mitral valve degeneration. J Vet Cardiol 2012;14:7–17.
16. Aupperle H, Maerz I, Thielebein J, et al. Immunohistochemical characterization of the extracellular matrix in normal mitral valves and in chronic valve disease (endocardiosis) in dogs. Res Vet Sci 2009;87:277–283.
17. Buchanan JW. Chronic valvular disease (endocardiosis) in dogs. Adv Vet Sci Comp Med 1977;21:75–106.
18. Disatian S, Ehrhart EJ III, Zimmerman S, Orton EC. Interstitial cells from dogs with naturally occurring myxomatous mitral valve disease undergo phenotype transformation. J Heart Valve Dis 2008;17:402–411.
19. Oyama MA, Chittur SV. Genomic expression patterns of mitral valve tissues from dogs with degenerative mitral valve disease. Am J Vet Res 2006;67:1307–1318.
20. Connelly JM, Bakay MA, Fulmer JT, et al. Fenfluramine disrupts the mitral valve interstitial cell response to serotonin. Am J Pathol 2009;175:988–997.
21. Jian B, Xu J, Connelly J, et al. Serotonin mechanisms in heart valve disease I: Serotonin-induced up-regulation of transforming growth factor-beta 1 via G-protein signal transduction in aortic valve interstitial cells. Am J Pathol 2002;161:2111–2121.
22. Rabkin E, Aikawa M, Stone J, et al. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. Circulation 2001;104:2525–2532.
23. Disatian S, Orton EC. Autocrine serotonin and transforming growth factor beta 1 signaling mediates spontaneous myxomatous mitral valve disease. J Heart Valve Dis 2009;18:44–51.
24. Disatian S, Lacfera C, Orton EC. Tryptophan hydroxylase 1 expression is increased in phenotype-altered canine and human degenerative myxomatous mitral valves. J Heart Valve Dis 2010;19:71–78.
25. Lacfera CM, Macrea HB, Kisiday JD, Orton EC. Static and cyclic tensile strain induce myxomatous effector proteins
and serotonin in canine mitral valves. J Vet Cardiol 2012; 14:223–230.

26. Scruggs SM, Disatian S, Orton EC. Serotonin transmembrane transporter is down-regulated in late-stage canine degenerative mitral valve disease. J Vet Cardiol 2010;12:163–169.

27. Arndt JW, Reynolds CA, Singletary GE, et al. Serum serotonin concentrations in dogs with degenerative mitral valve disease. J Vet Intern Med 2009;23:1208–1213.

28. Ni W, Watts S. 5-hydroxytryptamine in the cardiovascular system: Focus on the serotonin transporter (SERT). Clin Exp Pharmacol Physiol 2006;33:575–583.

29. Ljungvall I, Hoglund K, Lilliehook I, et al. Serum serotonin concentration is associated with severity of myxomatous mitral valve disease in dogs. J Vet Intern Med 2013;27:1105–1112.

30. Hansson K, Haggstrom J, Kvart C, Lord P. Left atrial to aortic root indices using two-dimensional and M-mode echocardiography in the dog and cat. Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. J Vet Intern Med 1993;7:247–252.

31. Thomas WP, Gaber CE, Jacobs GJ, et al. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. J Vet Intern Med 1993;7:247–252.

32. Rouzaud-Laborde C, Hanoun N, Baysal I, et al. Role of endothelial AADC in cardiac synthesis of serotonin and nitrates accumulation. PLoS One 2012;7:e34893.

33. Kema IP, de Vries EG, Schellings AM, et al. Improved diagnosis of carcinoid tumors by measurement of platelet serotonin. Clin Chem 1992;38:534–540.

34. Nigmatullina RR, Kirillova VV, Pourjikiya RK, et al. Disrupted serotonergic and sympathoadrenal systems in patients with chronic heart failure may serve as new therapeutic targets and novel biomarkers to assess severity, progression and response to treatment. Cardiology 2009;113:277–286.

35. Mezzano D, del Pino GE, Montesinos M, et al. Platelet 5-hydroxytryptamine increases with platelet age in dogs. Thromb Haemost 1991;66:254–258.

36. Sonobe T, Akiyama T, Du CK, et al. Contribution of serotonin uptake and degradation to myocardial interstitial serotonin levels during ischaemia-reperfusion in rabbits. Acta Physiol (Oxf) 2013;207:260–268.

37. Xu Y, Huo Y, Toufektsian MC, et al. Activated platelets contribute importantly to myocardial reperfusion injury. Am J Physiol Heart Circ Physiol 2006;290:H692–H699.

38. Shimizu Y, Minatoguchi S, Hashimoto K, et al. The role of serotonin in ischemic cellular damage and the infarct size-reducing effect of sarpogrelate, a 5-hydroxytryptamine-2 receptor blocker, in rabbit hearts. J Am Coll Cardiol 2002;40:1347–1355.

39. Tarnow I, Kristensen AT, Texel H, et al. Decreased platelet function in Cavalier King Charles Spaniels with mitral valve regurgitation. J Vet Intern Med 2003;17:680–686.

40. Tanaka R, Yamane Y. Platelet aggregation in dogs with mitral valve regurgitation. Am J Vet Res 2000;61:1248–1251.

41. Cowan SM, Bartges JW, Gompf RE, et al. Giant platelet disorder in the Cavalier King Charles Spaniel. Exp Hematol 2004;32:334–350.

42. Olsen L, Kristensen A, Haggstrom J, et al. Increased platelet aggregation response in Cavalier King Charles Spaniels with mitral valve prolapse. J Vet Intern Med 2001;15:209–216.

43. Tarnow I, Kristensen A, Olsen L, et al. Dogs with heart diseases causing turbulent high-velocity blood flow have changes in platelet function and von Willebrand factor multimer distribution. J Vet Intern Med 2005;19:515–522.