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

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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).

Animals: Forty-five dogs comprised 4 plasma groups of Cavalier King Charles Spaniels (CKCS) or non-CKCS, either healthy (CON) or MMVD affected: CKCS CON (n = 12); non-CKCS CON (n = 8); CKCS MMVD (n = 14); non-CKCS MMVD (n = 11). Twenty-four dogs comprised 3 tissue groups: MMVD (n = 8); other-HD (heart disease) (n = 7); non-HD, extracardiac disease (n = 9).

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 (11.9 ng/mg; range, 4.0–104.8) versus other-HD (0.9 ng/mg; range, 0–10.1; $P = .011$) and non-HD (2.5 ng/mg; range, 0–6.9; $P = .001$).

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.[1] 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.[1–5] 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.[6] Exogenous 5HT administration to

Abbreviations:

5HT	5-hydroxytryptamine
αSMA	alpha-smooth muscle actin
ARVC	arrythmogenic 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

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rats also has been linked to valvular heart disease.[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.[9] Several lines of evidence support a role for 5HT in the pathogenesis of canine myxomatous mitral valve disease (MMVD).[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.[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.[18,19] Serotonin has been linked to VIC activation in several species, including humans, rats, and dogs,[20–23] and several studies have shown altered local 5HT signaling in canine myxomatous mitral valves.[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[24] and (2) increased platelet-derived serum 5HT concentration, primarily in dogs with early stages of MMVD.[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.[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 $5HT_{2A}R$.[9] Increased myocardial fibrosis and arterial changes have been reported in dogs with congestive heart failure (CHF) because of MMVD.[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[31] were performed by cardiologists or trainees under the direct supervision of a cardiologist, using an echocardiographic unit[a] with 3S and 5S transducers or a unit[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[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.[c] Diastolic left atrial (LAD) and aortic (Ao) diameter and systolic and diastolic left ventricular diameters (LVIDs and LVIDd, respectively) were indexed to body weight according to previously published formulas[33]: $iLAD = LAD/body\ weight\ (BW)^{0.345}$, $iAo = LVIDs/BW^{0.341}$, $iLVIDs = LVIDs/BW^{0.315}$, and $iLVIDd = LVIDd/BW^{0.294}$.

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 × *g* for 3 minutes. The supernatant PRP was collected and stored at −80°C. Remaining plasma was centrifuged at 3,000 × *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.[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 cm^3 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.[34] Briefly, 5HT was extracted from PRP and PPP with Chromsystems reagent using an Internal Standard.[e] Extracted 5HT was quantified electrochemically[f] 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[g] 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.[h] Overall differences among groups were analyzed using Kruskal-Wallis, and if significance was found, Wilcoxon rank sum tests were used for posthoc analyses. Data tabulated as counts were analyzed using Fisher's exact test. Associations of 5HT concentration with age, weight, ISACHC class, LA/Ao, iLVIDd, 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)^{3,4}	10.5 (4–15)^{1,2}	9 (7–14)^{1}
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	0/0/3/6/2
iLVIDd (mm)	N = 45	1.51 (1.17–1.66)	1.37 (1.23–1.65)^{3,4}	1.90 (1.41–2.63)^{1}	1.98 (1.61–2.38)^{1}
iLVIDs (mm)	N = 45	0.99 (0.71–1.15)	0.94 (0.72–1.21)^{3}	1.1 (0.86–1.78)	1.03 (0.70–1.36)
iLAD (mm)	N = 45	0.98 (0.92–1.26)	1.00 (0.77–1.22)^{3,4}	1.33 (0.94–2.11)^{1}	1.40 (1.21–2.01)^{1}
iAoD (mm)	N = 45	0.77 (0.59–0.92)	0.74 (0.64–0.91)	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)^{3,4}	1.83 (1.11–2.82)^{1}	2.07 (1.60–3.12)^{1}
MV E (m/s)	N = 27	0.61 (0.44–0.70)	0.65 (0.61–0.83)^{4}	0.74 (0.49–1.38)^{3}	1.23 (1.04–1.66)^{1}
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)
Plt EDTA (/μL)	N = 45	333.5 (218–693)	292.5 (156–522)	326.5 (133–439)	396 (237–529)
Plt PRP (/μL)	N = 45	440 (159–1191)	363 (112–551)	486.5 (134–613)	342 (126–530)
Plt 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 (Plt) in EDTA whole 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 CON[1], non-CKCS CON[2], CKCS MMVD[3], and non-CKCS MMVD[4]. Superscripts that are not italicized indicate that the respective P is <.05 and italicized superscripts indicate that the respective P-value is <.01.

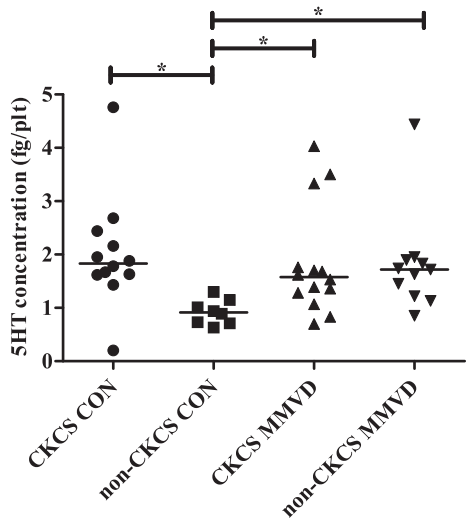

Fig 1. Serotonin (5-hydroxytryptamine, 5HT) concentration of platelets from platelet-rich plasma from Cavalier King Charles Spaniels (CKCS) and non-CKCS without (CON) and with myxomatous mitral valve disease (MMVD). Groups: CKCS CON (n = 8); Non-CKCS CON (n = 12); CKCS MMVD (n = 14); and non-CKCS MMVD (n = 11). **P*-value from Wilcoxon rank sum test <.05.

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 ($\chi^2 = 0.785$, $P = .27$), 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 arrythmogenic 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;

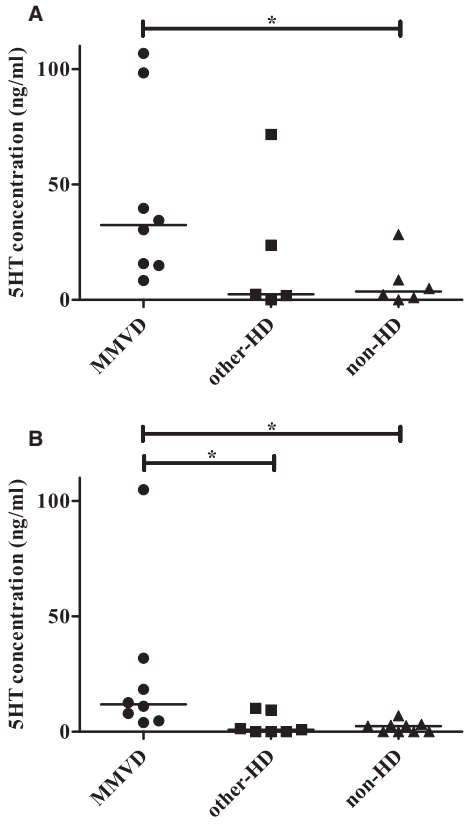

Fig 2. Serotonin (5-hydroxytryptamine, 5HT) concentration in mitral valve (MV) leaflet **(A)** and left ventricular (LV) myocardium **(B)** in 3 different dog groups: myxomatous mitral valve disease (MMVD) (n = 8); other-HD, non-MMVD cardiac disease (n = 7), and non-HD, no heart disease (n = 9). Because of technical difficulties, MV 5HT concentration from 5 dogs could not be determined. **P*-value from Wilcoxon rank sum test <.05.

$P = .01$), but not higher than 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[27,28] 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.[35] 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.[35] In a study of human patients with chronic CHF, platelet 5HT concentration was increased 3.5 to 18-fold over controls,[36] 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,[27,28] 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,[28] 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.

Mitral 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.[23] The present study revealed significantly higher LV 5HT concentrations in MMVD dogs versus both noncardiac conditions and dogs with DCM/ARVC, which suggests either local 5HT production within the myocardial tissue or exposure to platelet-derived 5HT.[9] 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.[37–39] Platelet lysate, and in particular platelet-derived 5HT, induces activation, migration, and proliferation of cardiac fibroblasts in vitro,[9] all of which are important steps in tissue remodeling. After injury, antagonism of the $5HT_{2A}R$ significantly decreases infarct size.[40] Previous studies indicated platelet dysfunction in CKCS[41] and other dogs breeds with MMVD,[42] but the exact nature and role of these abnormalities is controversial.[42–45] 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, $5HT_{2B}R$, and TGFβ1 in mildly MMVD-affected valves.[23,24,26]

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

PRP was from 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, Broendby, Denmark
[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, Gentofte, Denmark; scil Animal Care; Gurnee, IL
[e] Chromsystems Instruments & Chemicals, Gräfelfing, Germany
[f] ESA. Coulochem III; Eurosep instruments, Cergy, France
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