Insights into Serotonin Signaling Mechanisms Associated with Canine Degenerative Mitral Valve Disease

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Little is known about the molecular abnormalities associated with canine degenerative mitral valve disease (DMVD). The pathology of DMVD involves the differentiation and activation of the normally quiescent mitral valvular interstitial cell (VIC) into a more active myofibroblast phenotype, which mediates many of the histological and molecular changes in affected the valve tissue. In both humans and experimental animal models, increased serotonin (5-hydroxytryptamine, 5HT) signaling can induce VIC differentiation and myxomatous valve damage. In canine DMVD, numerous lines of evidence suggest that 5HT and related molecules such as transforming growth factor-β play a critical role in the pathogenesis of this disease. A variety of investigative techniques, including gene expression, immunohistochemistry, protein blotting, and cell culture, shed light on the potential role of 5HT in the differentiation of VIC, elaboration of myxomatous extracellular matrix components, and activation of mitogen-activated protein kinase pathways. These studies help support a hypothesis that 5HT and its related pathways serve as an important stimulus in canine DMVD. This review describes the pathological characteristics of canine DMVD, the organization and role of the 5HT pathway in valve tissue, involvement of 5HT in human and experimental models of valve disease, avenues of evidence that suggest a role for 5HT in naturally occurring DMVD, and finally, a overarching hypothesis describing a potential role for 5HT in canine DMVD.

Key words: Cellular biochemistry; Molecular biology; Physiology; Valvular disease.

Degenerative mitral valve disease (DMVD) is the most common heart disease of dogs, and despite working knowledge of the gross, histological, and echocardiographic characteristics of the disease, very little is known about the molecular mechanisms that underlie its pathophysiology. In humans1–9 and animals,10–17 serotonin (5-hydroxytryptamine, 5HT) and 5HT-related pathways have been associated with myocardial, vascular, and valvular disease, and emerging evidence points toward a role of 5HT in naturally occurring canine DMVD. The purpose of this review is to describe (1) the organization and putative functions of the 5HT system in cardiac tissue, (2) involvement of 5HT mechanisms in human and animal models of valve disease, (3) involvement of 5HT mechanisms in dogs with spontaneously occurring DMVD, (4) an overarching hypothesis for the role of 5HT in canine DMVD, and (5) future directions of study to better elucidate the role of 5HT in DMVD.

Pathophysiologic Features of Canine DMVD

DMVD is present in approximately one third of all dogs over the age of 10 years and is the most frequent cause of congestive heart failure in dogs.18,19 DMVD is most prevalent in small dogs, and breeds such as the Cavalier King Charles Spaniel, Chihuahua, Maltese, toy and miniature Poodle, and Pekingese are highly predisposed.20 The natural history of the disease is one of adult onset, variable progression with aging, and eventual development of congestive heart failure in dogs with severe disease. In Cavalier King Charles Spaniels, the time from onset of a heart murmur to death because of congestive heart failure can be quite accelerated, highlighting the rapid nature of disease progression in this particular breed. DMVD causes incompetence of the mitral valve and mitral regurgitation, which promotes sodium and water retention, activation of neurohormonal systems, volume overload, and eventual congestive heart failure. Current treatment for DMVD with diuretics, vasodilators, and positive inotropes centers on symptomatic relief rather than arresting progression of disease.

The pathologic features of DMVD have been described extensively,19,21–23 and this review will focus on the most salient features. Affected valve leaflets are grossly thickened and opaque with areas of nodule formation along the free edges. As disease progresses, areas of coalesced nodules and leaflet thickening occupy large portions of the valve surface. Chordae tendineae become thickened, elongated, or ruptured, and portions of the valve leaflet subsequently may bulge or prolapse toward the atrial chamber. Histologically, the mitral valve is comprised of 4 distinct layers, each of which is affected by DMVD. The atrialis is made up of mostly
elastic fibers with occasional collagen I and III fibers scattered throughout. The spongiosa layer is predominantly comprised of proteoglycans with a modest amount of collagen, and is sparsely populated by fibroblast-like cells called valvular interstitial cells (VIC). The main structural layer of the valve, the fibrosa, contains densely arranged collagen fibers, few elastic fibers, and extends into the chordae tendineae that are attached to the valve leaflet edge. The final layer, the ventricularis, is comprised of collagen fibers and serves as the boundary between the fibrosa and the endothelial surface of the ventricular side of the valve. Covering the surface of the valve leaflets is the 2nd predominant valvular cell type, the valvular endothelial cell. Characteristic histologic changes occur in valves affected by DMVD (Fig 1). Mild disease is typified by an increase in the extracellular matrix (ECM) component of the atrialis, loss of collagen organization in the fibrosa and proliferation of valvular endothelial cells. As disease progresses, nodules of proteoglycan and basement membrane material form, enlarge, and coalesce, and the collagen arrangement in the fibrosa becomes increasingly disordered, fragmented, and lacking. In the most severely affected valves, the normal architecture of the valve is completely destroyed with prolific deposition of ECM within the spongiosa, disorganization of collagen fibers in the fibrosa, and denudation of the endothelial covering of the valve surface.

As previously indicated, 2 cell types predominate in valve tissue, the valvular endothelial cell, which provides a smooth nonthrombogenic surface to the valve, and the fibroblast-like VIC, which maintains the ECM, elastin, and collagen components of the spongiosa and fibrosa layers. In normal valve tissue, the VIC is relatively quiescent, and cell numbers are sparse and distributed evenly within the spongiosa layer. In DMVD, the VIC undergoes a phenotype transformation into a more active myofibroblast. The hallmark of myofibroblast differentiation is the expression of smooth muscle actin and vimentin. Activated myofibroblasts are an important component of canine DMVD. Myofibroblasts mediate the simultaneous expansion of the spongiosa and destruction of the fibrosa by their expression of smooth muscle actin, collagenases, fibronectin, matrix metalloproteinases, chondroitin sulfate, cathepsin D, glycosaminoglycans, and other molecules. A variety of biochemical and mechanical stimuli can induce the activation of myofibroblasts and resultant deposition of myxoid material within the valve. Thus, VIC differentiation and the myxomatous remodeling process represent 1 final common pathway arising from valve injury or aging (another potential pathway being valve calcification). The molecular signals that mediate VIC differentiation and proliferation include transforming growth factor β1 (TGF-β1), platelet-derived growth factor, fibroblast growth factor, thrombin, fibronectin, and vascular adhesion factors. Endothelial-derived nitric oxide and endothelin-1 likely also play an important role in disease. In summary, a key step in the myxomatous degenerative process is activation of VIC into myofibroblasts and induction of proliferative signaling pathways that result in abnormal regulation of both the ECM and the structural integrity of the valve.

Organization and Role of 5HT in the Cardiovascular System

5HT is a monoamine neurotransmitter that is produced in the central nervous system as well as in enterochromaffin cells in the gastrointestinal tract. In some lower species of animals, 5HT serves as the main excitatory neurotransmitter. In higher mammalian species, 5HT has been largely replaced by norepinephrine, but 5HT continues to play an important role in mood, appetite, metabolism, vasoconstriction, and platelet function. In nonneuronal tissue, 5HT is produced from tryptophan in a 2-step process wherein hydroxylation by tryptophan hydroxylase-1 is the rate-limiting step. Central production of 5HT is regulated by transport of tryptophan across the blood–brain barrier, whereas in the periphery, the majority of 5HT is both produced, and stored within the enterochromaffin cells. Upon release into the circulation, 5HT is rapidly taken up by platelets via the 5HT transporter (5HTT, or alternatively,
5HT-reuptake transporter [SERT]) and virtually all circulating 5HT is stored in the dense granules of platelets. Platelet 5HT release is triggered by a variety of stimuli, including endothelial damage, platelet aggregation, and 5HT receptor (5HT-R) agonists. High concentrations of SERT also are found in the pulmonary and coronary endothelium and likely contribute to regulation of local vascular tone. Interestingly, SERT is expressed in rat and mice embryonic myocardium and is specifically involved in valvulogenesis. In the mature rodent, heart SERT persists in coronary endothelium and valvular tissues. The precise function of 5HT in valve tissue is unknown, but based on its role in valve formation and its expression on mature valves, it is likely that both 5HT and SERT play a physiological role in maintaining healthy valve tissue. 5HT is taken up by SERT and metabolized by monoamine oxidase into 5-hydroxyindole acetic acid, which then is excreted into the urine. In this way, SERT acts as the primary clearance mechanism for locally produced or released 5HT at the level of the mitral valve.

In cardiovascular tissues, 5HT mediates its actions through at least 5 different 5HT-R families, each comprised of a variety of receptor subtypes. 5HT-R1A are located in the central nervous system and regulate cardiac sympathetic and vagal tone, whereas 5HT-R1B and 5HT-R2A are widely distributed throughout vascular endothelium and smooth muscle where they induce vasoconstriction. Human valve tissue is rich in 5HT-R and expresses 5HT-R2B as well as 5HT-R1B, 5HT-R1D, and 5HT-R2A. 5HT-R3 are located on afferent vagal and sympathetic nerve terminals and mediate reflex bradycardia, whereas 5HT-R4 are found in atrial and ventricular tissue and increase contractility. Finally, 5HT-R2 are found in coronary artery smooth muscle and are thought to provoke vasodilatation. Thus, the cardiovascular system is rich in its ability to bind and respond to 5HT.

The canine cardiovascular system is similarly rich in 5HT-signaling components. In particular, canine mitral valve tissue expresses 5HT-R2A, 5HT-R2B, 5HT-R1B, as well as SERT and tryptophan hydroxylase. 5HT-R2A and 5HT-R2B are secondarily linked to Gq proteins, and through activation of phospholipase C these receptors mediate downstream mitogen-activated protein kinase signaling pathways such as the extracellular signal-regulated kinase 1 (ERK1) and 2 (ERK2) systems. Phosphorylation of ERK1/2 initiates processes of cellular differentiation and proliferation. Importantly, in valve tissue, ERK1/2 transforms normally quiescent VIC into the more active myofibroblast phenotype. The 5HT pathway also is closely linked to the TGF-β1 system. 5HT increases TGF-β1 expression and is closely associated with differentiation and proliferation of VIC. Thus, both the 5HT and TGF-β1 systems are found in the canine mitral valve, and by their ability to activate myofibroblasts, these systems may play an important role in the development and progression of DMVD. Further insight into this hypothesis is gained by examining the role of 5HT in both humans as well as in experimental animal models.

### 5HT Mechanisms in Human and Animal Models of Valve Disease

The classic model of 5HT-mediated valve involves humans afflicted with carcinoid tumors. These tumors, typically within the intestinal tract and liver, secrete large concentrations of free 5HT into the circulation, and expose the systemic venous circulation to excessive amounts of 5HT. Affected humans demonstrate “carcinoid syndrome,” which is characterized by cutaneous flushing, intestinal hypermotility, and in 40% of patients, development of pulmonary and tricuspid valve disease. Carcinoid-mediated valve disease involves formation of distinctive “carcinoid” plaques of fibrous and ECM on the valve surfaces of the right side of the heart. Grossly, the leaflets are markedly thickened and opaque. Histologically, valves demonstrate VIC proliferation and differentiation, collagen deposition, and excessive proteoglycan deposition and remodeling within the extracellular space. These changes bear much similarity to degenerative myxomatous disease in both humans and dogs. In approximately 10% of patients with carcinoid disease, changes also occur on the mitral or aortic valves. The lower incidence of left-sided disease likely is because of clearance of 5HT by monoamine oxidase located within the pulmonary vascular endothelium. Although the morphologic and molecular characteristics of human 5HT-mediated valve disease and canine DMVD are not identical, they share some intriguing common traits, including activation of VIC, production of myxoid ECM, and upregulation of 5HT and TGF-β1 signaling pathways. In humans with carcinoid syndrome, the development of valve disease is clinically relevant and contributes to overall mortality. In dogs, carcinoid tumors are very rare and in the few reported cases, the prevalence of valve disease was not specifically reported.

Further evidence that 5HT signaling can mediate valve disease stems from use of serotonergic drugs, including the anorectic drug combination fenfluramine-phenteramine, ergot derivatives used for treatment of Parkinson’s disease such as pergolide, ergot derivatives used for treatment of migraine headaches such as methysergide, as well as the illicit recreational drug 3,4-methylenedioxymethamphetamine (MDMA or “Ecstasy”) and the associated development of myxomatous valve degeneration. In humans, use of fenfluramine-phenteramine was associated with a risk factor of 2.2 and 1.6 for incidence of aortic regurgitation and mitral regurgitation, respectively, and long-term use of pergolide and methysergide produces valve lesions on both the tricuspid and mitral valves. Although the exact mechanisms surrounding disease are not entirely clear, there is general consensus that 5HT-R2B agonism is the most likely cause. Evidence for 5HT-mediated valve disease is further provided by studies of rats that received daily injections of 5HT and developed valvulopathies and VIC activation. Droogman et al reported that pergolide administration induced mitral valve disease in rats and Hauso et al reported that this phenomenon could be prevented by coadministration of...
a 5HT-R2B/2C blocker. SERT knock-out mice develop myocardial fibrosis and valvulopathy presumably because of decreased clearance of 5HT in the cardiac vascular endothelium and valve tissues.15

5HT Mechanisms in Dogs with DMVD

A role for 5HT mechanisms in canine DMVD is suggested by several avenues of investigation, including transcriptional, immunohistochemical, cell culture, and protein studies. An oligonucleotide microarray study from our laboratory indicated a nearly 4-fold increase in the transcription of 5HT-R2B in affected canine mitral valve tissue as compared with control.47 Moreover, transcription of several downstream components of both the 5HT and TGF-β systems also was found to be upregulated.47 Immunohistochemical and immunoblot analysis of degenerative valve tissue indicates increased staining and protein concentration for 5HT-R2B and tryptophan hydroxylase 1 as well as for latent TGF-β1 and TGF-β receptors I and II (Fig 2A,B).48 Both Disatian and Orton48 and Connolly et al65 have reported activation of the mitogen-activated pathway ERK1/2 in canine mitral tissues, suggesting that increased 5HT signaling contributes to activation of VIC.

Experiments involving cultured human and canine mitral VIC in our laboratory provide further insight. Both human and canine VIC demonstrate increased ERK1/2 phosphorylation when exposed to exogenous 5HT (Fig 2B).65 Interestingly, this response could be potentiated by coinoculation with either the serotonergic agent fenfluoramine or the SERT inhibitor fluoxetine (Paxil, GlaxoSmithKline, Philadelphia, PA) (Fig 3). These experiments suggest that either increased 5HT signaling or decreased 5HT clearance can activate mitogenic pathways in canine VIC.

Moreover, 5HT appears capable of directly stimulating VIC into the more active myofibroblast phenotype. Canine VIC incubated with 5HT demonstrate significantly increased radiometric indices of proliferation ([3H]-thymidine), ECM production ([3H]-glucosamine), and collagen deposition ([3H]-proline) (Fig 4).65 In both human26,27 and canine24 valve disease, the phenotype switch from VIC to myofibroblast is a hallmark of valve injury and remodeling, thus the ability of 5HT to induce such a switch is very intriguing. Interestingly, activation of canine VIC could be inhibited by coinoculation with either ketanserin, a 5HT-R2A receptor blocker, or GR55562, a 5HT-R1B receptor blocker, indicating that these 2 receptor types are potentially involved in 5HT-induced changes (Fig 5).65

The mitogenic and proliferative responses of VIC usually are viewed as complementary and simultaneous but the interaction between cell number and cell activity may not be entirely linked. For instance, the serotonergic drug fenfluramine appears to favor the production of ECM while simultaneously inhibiting the mitogenic response to 5HT.65

Putative sources of heightened 5HT signaling in dogs with DMVD include increased circulating or platelet-derived 5HT,66 increased autocrine 5HT production,48 or...

Fig 2. Western blots of mitral valvular interstitial cells. (A) Blots of interstitial cells from normal (N) and myxomatous (M) canine mitral valves for latent transforming growth factor β1 (latent TGF-β1) and TGF-β receptors I (TGFβ RI) and II (TGFβ RII). Control lane for a-tubulin is also shown. Reprinted with permission from Disatian and Orton.48 (B) Representative Western blots of human (top) and canine (bottom) myxomatous mitral valvular interstitial cells showing dose-dependent responsiveness to exogenous 5-hydroxytryptamine (5HT) for extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation (activation). 5HT concentrations from $10^{-9}$ to $10^{-5}$ M were studied. Reprinted from Am J Pathol 2009,175:988–99765 with permission from the American Society for Investigative Pathology.

Fig 3. Representative Western blots of canine mitral valvular interstitial cells demonstrating that coinoculation with 5-hydroxytryptamine (5HT) and either the serotonergic agent fenfluramine (A) or the serotonin reuptake transporter (SERT) blocking agent fluoxetine (B) increases extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation (activation). Fenfluramine or fluoxetine concentrations from $10^{-9}$ to $10^{-3}$ M were studied. Reprinted from Am J Pathol 2009,175:988–99765 with permission from the American Society for Investigative Pathology.
valvular SERT deficiency.\textsuperscript{48} We have previously reported increased serum 5HT concentrations in dogs with DMVD.\textsuperscript{66} Serum 5HT was increased by approximately 50\% in dogs with DMVD as well as in small breed dogs predisposed to DMVD as compared with healthy large breed control dogs. Interestingly, Cavalier King Charles Spaniels, which are highly predisposed to DMVD as well as macrothrombocytosis, had significantly higher serum 5HT concentrations than did other breeds of dogs.\textsuperscript{66} In general, measurement of serum 5HT encompasses 2 different sources, platelet-stored 5HT and plasma 5HT, of which platelet 5HT is the much larger source. The hypothesis that platelet-derived 5HT directly activates myocardial or valvular fibroblasts is supported by Yabanoglu et al\textsuperscript{67} who reported chemotaxis of platelets to regions of cardiac damage, with subsequent 5HT release, fibroblast differentiation, and production of TGF-\(\beta\) and matrix metalloproteinases. This effect was inhibited by coadministration of ketanserin, a 5HT–R\(2\alpha\)-specific blocking agent, again suggesting that this receptor plays an important role in the activation of cardiac fibroblasts by 5HT. This study\textsuperscript{67} is important insofar as it supports a key hypothesis involving direct interaction among platelets, platelet-derived 5HT, 5HT receptors, increased 5HT and TGF-\(\beta\) signaling, and subsequent cardiac cell differentiation.

Affected canine mitral valve leaflets display abnormal and denuded endothelium\textsuperscript{47} (Fig 6) and express a variety of inflammatory cell adhesion molecules such as intracellular and vascular cell adhesion molecule-1.\textsuperscript{47} These alterations are likely to attract platelets to the valve surface, and scanning electron microscopy studies of affected canine\textsuperscript{68} and human\textsuperscript{69,70} mitral valves show small numbers of platelets adhered to areas of endothelial damage. The aggregation potential of platelets in dogs with DMVD has been variably reported as decreased\textsuperscript{71} or increased\textsuperscript{72} by different investigators, and additional studies are needed to better define if, how, and to what extent platelet adhesion, aggregation, and 5HT release play a role in the initiation or progression of valve injury.

Disatian and Orton\textsuperscript{48} reported increased concentrations of tryptophan hydroxylase in myxomatous canine mitral valves suggesting increased local (autocrine) production of 5HT. The same study\textsuperscript{48} also reported decreased staining and protein concentration of the 5HT uptake transporter SERT in affected dogs as compared with controls (Fig 7). Increased autocrine production of 5HT coupled with the potential for decreased local 5HT uptake and metabolism by SERT is a highly intriguing combination of findings, particularly considering that mice deficient in the SERT gene develop cardiac fibrosis and valvulopathy.\textsuperscript{15} Expression and activity of the SERT protein in humans are influenced by polymorphisms in the SERT gene \textit{SLC6A4},\textsuperscript{73,74} and these polymorphisms likely influence susceptibility to adverse effects of 5HT signaling.\textsuperscript{75} Specifically, a 44-bp insertion or deletion polymorphism in the promoter region of \textit{SLC6A4} regulates transcriptional efficiency, with
the short (deletion) variant demonstrating decreased SERT expression by 40–70% and decreased SERT protein by 30–40%. Platelets, being rich in SERT, demonstrate differential rates of 5HT uptake depending on the insertion or deletion genotype, and the presence of this and other SERT polymorphisms in dogs warrant additional study.

Studies involving TGF-β1 provide an indirect line of evidence that further suggests a role for increased 5HT signaling in canine DMVD. Transcriptional, immunohistochemical, and protein studies of dogs, humans, and animal models indicate a role for TGF-β1 in the development of and pathological response to valve disease. TGF-β1 has been shown to be a potent stimulus for transformation of VIC into myofibroblasts, and Aupperle et al. report that myofibroblasts from diseased canine mitral valves strongly express TGF-β1 and β3. TGF-β1 is part of a final common pathway that is triggered by a variety of different injurious factors, including direct trauma, cyclic tension, shear stress, and increased 5HT signaling. An important model of TGF-β1–mediated disease is Marfan syndrome. Marfan syndrome is an inherited disease caused by deficiency of the ECM protein fibrillin-1, and with regard to cardiac involvement, is associated with mitral valve prolapse, myxomatous degeneration, and aortic aneurysms. A close relationship between decreased fibrillin-1 and increased TGF-β1 signaling is important in the pathogenesis of this disease, and suppression of TGF-β1 using either angiotensin-II receptor antagonists or anti–TGF-β1 antibodies is of great therapeutic interest. Thus, Marfan syndrome may
represent a human condition similar to canine 5HT/TGF-β1–mediated disease, and treatments that are effective in humans with Marfan syndrome may have application in dogs.

**Integrated Hypothesis Involving 5HT and Canine Mitral Valve Disease**

Based on available data, a hypothesis regarding the role of 5HT in canine DMVD is proposed (Fig 8). Valve injury is initiated by an unknown etiology that may involve genetic, environmental, toxic, nutritional, or metabolic factors. Although possible, circulating, autocrine, or dysregulated 5HT signaling is less likely to be the sole primary cause of disease because of differences in the specific histopathologic findings in carcinoid disease (eg, carcinoid plaques) as compared with canine DMVD. After injury and development of valvular regurgitation, sheer stress, and mechanical deformation of the valve surface damage valvular endothelial cells, exposing underlying collagen, and eliciting the expression of surface adhesion molecules. Platelets, rich in 5HT, are attracted to the valve surface, aggregate, and stimulate local 5HT production and release. Autocrine production of 5HT also is increased, and through positive feedback from mechanical stress and other 5HT-related stimulatory mitogenic pathways, contributes to increased 5HT signaling. Within valve tissue, 5HT binds to specific 5HT receptors (likely 5HT-R2A, 5HT-R2B, or 5HT-R1B) located on VIC, thereby leading to activation of intracellular mitogenic pathways, including ERK1/2. VIC subsequently are induced to transform from their normal quiescent phenotype into activated myofibroblasts. These activated myofibroblasts mediate pathologic valve remodeling by increased deposition of glycosaminoglycans, collagen turnover, and expression of TGF-β1 and other molecules such as fibronectin and matrix metalloproteinases. In breeds predisposed to DMVD, increased circulating, platelet, or autocrine 5HT or dysregulation of the normal valvular 5HT-SERT system may increase susceptibility of the valve to development of clinically relevant disease after the inciting injury. If 5HT clearance mechanisms such as SERT are decreased either by decreased expression or polymorphisms, the propensity for enhanced local 5HT signaling, and further valve injury is increased. Thus, our hypothesis proposes that 5HT (and TGF-β1)–mediated mechanisms represent a final common pathway for inciting injury and eliciting pathologic remodeling of the valve, with development of valvular incompetence and additional valve injury. Interruption of this pathway may have important therapeutic implications in the development and progression of DMVD.

**Future Directions of Study Involving 5HT and Canine DMVD**

To fully understand the role and importance of 5HT in canine DMVD, additional studies are needed. These studies should use canine VIC culture techniques to further characterize the 5HT-receptor populations that mediate the molecular responses to in vitro 5HT exposure. The differentiation of primary etiology from secondary effects can be accomplished by studies com-

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**Fig 8.** Hypothesis involving heightened or dysregulated 5-hydroxytryptamine (5HT) signaling as a potential contributor to degenerative mitral valve disease in dogs. Local or circulating 5HT or dysregulation of the normal valvular 5HT system may represent an inciting cause of valve injury or contribute to a final common pathway involving valvular interstitial cell differentiation that follows the initial valve injury. Note the close relationship between 5HT and transforming growth factor-β (TGF-β) in mediating cell proliferation and differentiation.
paring animal models with spontaneous disease.\textsuperscript{94} The role of SERT in canine DMVD warrants further investigation, and findings may have importance for human medicine. Use of selective SERT inhibitors such as fluoxetine has not been associated with a higher incidence of valvular regurgitation, but the potential effects on heart valve disease are limited to the findings from a small cross sectional study involving only 292 human patients.\textsuperscript{95} Finally, clinical studies in the whole animal can help test and identify agents, such as 5HT-R antagonists, that may slow down, stop, or even reverse progression of disease. The effort to better understand the basic mechanisms of 5HT-mediated disease drives the potential for future collaborative and translational investigation by researchers across several fields of interest. For example, the discovery that ketanserin blocks ERK1/2 phosphorylation in canine VIC has prompted the authors to initiate pilot safety and tolerability studies utilizing this specific 5HT-R\textsubscript{2A} antagonist in dogs with naturally occurring DMVD.

In summary, although very little is known about the molecular mechanisms responsible for the development and progression of DMVD in dogs, several lines of evidence point toward the 5HT and TGF-\beta pathways as important in the activation of VIC and the molecular changes characteristic of this disease. Future studies should examine the role of 5HT signaling in both inducing and accelerating valve injury. A greater understanding of these mechanisms may yield insight into the etiology of disease, mechanisms of disease progression, and new therapies.

Footnotes


\textsuperscript{95} Connolly JM, Oyama MA, Gorman RC, et al. Serotonin transporter blockade with dexfenfluramine or fluoxetine increases serotonin-mediated ERK1/2 phosphorylation in heart valve interstitial cells: Implications for serotonin-related heart valve disease. Circulation 2006;114:III-300 (abstract)

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