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5-Hydroxytryptamine (5HT)-induced valvulopathy: Compositional valvular alterations are associated with 5HT2B receptor and 5HT transporter transcript changes in Sprague-Dawley rats

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Abstract

Several drugs have been linked to valvulopathy in humans, including therapeutic agents for obesity, Parkinson's disease and migraine. There is increasing evidence that the 5-hydroxytryptamine 2B receptor (5HT2BR) activation and/or increased circulating 5HT (5-hydroxytryptamine) may play a significant role in the pathogenesis of drug-induced valvulopathy. In the present study, we investigated whether 7-day 5HT subcutaneous injections led to structural and compositional abnormalities in conjunction with transcriptomic modulation of 5HT2BR and 5HT transporter (5HTT) genes in the aortic and mitral valves of Sprague-Dawley (SD) rats. Subcutaneous injections of 5HT for 7 days resulted in thickening and compositional alteration of aortic and mitral valves in SD rats. More specifically, valve-leaflets from 5HT-treated rats had greater valve thickness, a higher amount of glycosaminoglycans (GAGs) and a lower amount of collagen. The compositional alteration was associated with up-regulation and down-regulation of 5HT2BR and 5HTT genes, respectively. The present study strongly suggests that the activation of 5HT2BR and inhibition of 5HTT played a significant role in the pathogenesis of 5HT-induced valvulopathy in SD rats. Thus, these findings further highlight the necessity and/or utilization of animal models to screen potential valvular effects of serotonergic compounds.

Keywords: Valvulopathy; 5-Hydroxytryptamine 2B receptor; 5-Hydroxytryptamine transporter; Anorexigens; Compositional morphometry; Quantitative image analysis; Sprague-Dawley rats

Introduction

Valvular disease or valvulopathy is a main cause of morbidity and mortality in humans worldwide, requiring valve replacement surgery, the second most common heart operation performed in the US (Keane et al., 1993; Thom et al., 2006). A link has now been established between valvulopathy and treatment with drugs for diverse indications, including therapeutic agents for obesity (fenfluramine and dexfenfluramine), Parkinson's disease (pergolide and cabergoline) and migraine (ergotamine, dihydroergotamine and

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methysergide) (Connolly et al., 1997; Jick et al., 1998; Fitzgerald et al., 2000; Rothman et al., 2000; Rajamannan et al., 2001; Weissman, 2001; Pritchett et al., 2002; Newman-Tancredi et al., 2002; Nebigil and Maroteaux, 2003; Schade et al., 2007; Zanettini et al., 2007). These drugs have been shown to cause 5-hydroxytryptamine 2B receptor (5HT2BR) activation and/or increased circulating 5HT (5-hydroxytryptamine, also known as serotonin) levels (Fitzgerald et al., 2000; Rothman et al., 2000; Pritchett et al., 2002). 5HT has a direct mitogenic effect on the cardiac valvular subendocardial cells, and this mitogenic effect is mediated by 5HT receptors (Fanburg and Lee, 1997; Rajamannan et al., 2001). Recent studies implicated preferential activation of 5HT2BR as a key step in initiating drug-induced valvulopathy in humans. The critical role of 5HT2BR activation in the pathogenesis of valvulopathy is further highlighted by the observation that chemically similar drugs, such as lisuride and terguride that are agonist for 5HT2C and 5HT2A receptors, and antagonist for 5HT2BR, were not associated with valvulopathy (Jahnichen et al., 2005; Roth, 2007). Similarly, no increase in the risk of valvulopathy was observed in patients treated with non-ergot derived dopamine agonist, pramipexole which has low affinity to the human 5HT2BR (Millan et al., 2002; Schade et al., 2007; Zanettini et al., 2007). As a consequence, it has been strongly recommended to screen future candidate drugs with serotonergic activity and their metabolites at the 5HT2BR comprehensively before launching clinical trials (Rothman et al., 2000; Setola et al., 2003; Setola and Roth, 2005; Roth, 2007). We previously reported the immunostaining and quantitative transcript levels of 5HT2BR and 5HT1BR in normal valve-leaflets (mitral, aortic, tricuspid and pulmonary valves) of Sprague-Dawley (SD) rats and Cynomolgus monkeys (Elangbam et al., 2005). The 5HT receptor expression in four heart valves is comparable among Cynomolgus monkeys, rats and humans, and therefore, the potential exists to gain mechanistic insight to drug-induced valvulopathy from animal studies.

5-Hydroxytryptamine has a wide range of biological functions. In the brain, 5HT is a well-known neurotransmitter. But at the periphery, 5HT originating from the gastrointestinal tract (i.e., enterochromaffin cells) is stored in blood platelets and participates in intestinal motility, fluid secretion, coagulation and blood pressure homeostasis. The biological action of 5HT is mediated via a plasma membrane receptor system that has at least 15 cognate receptors divided into seven families, and all except one of these are G-protein-coupled receptor subfamilies (Nebigil and Maroteaux, 2003). 5HT transporter (5HTT) facilitates intracellular processing of 5HT after receptor interactions (Rothman and Baumann, 2002). 5HTT is responsible for 5HT uptake and subsequent inactivation of the amine passing through the lung, and reduced 5HT inactivation is one proposed mechanism of the valvulopathy observed in individuals treated with fenfluramine and dexfenfluramine. Recently, it has been shown that interference with 5HT transmembrane processing via knocking out 5HTT resulted in valvulopathy in mice, establishing a link between 5HTT and the development of cardiac fibrosis and valvulopathy in vivo. Absence of transmembrane processing (via knocking out 5HTT), may result in increased and persistent 5HT receptor interactions, and increased valvular mitogenic activity and extracellular matrix production in the 5HTT-knockout mice (Mekontso-Dessap et al., 2006). Further, an association between spontaneous mitral valvulopathy (SMV) and increased number of 5HT2BR-positive cells in valve-leaflets has been described in SD rats, suggesting that 5HT2BR may play a role in its pathogenesis (Elangbam et al., 2006). 5HT2BR may also play a role in the exacerbation of SMV in Fischer 344 rats by DL-amphetamine treatment (Elangbam et al., 2006). Amphetamine derivatives such as 3,4-methylenedioxymethamphetamine (MDMA, ''Ecstasy'') and 3,4-methylenedioxyamphetamine (MDA) have been shown to induce prolonged mitogenic response in human valvular interstitial cells through in vitro activation of 5HT2BR, similar to those induced by fenfluramine in vivo (Setola et al., 2003).

Valvular lesions associated with anti-obesity drugs (anorexigens) exhibit distinctive microscopic features in humans. McDonald et al. (2002) have demonstrated that anorexigen-exposed valves contain more glycosaminoglycans (GAGs) than normal or floppy valves. In humans, normal valves are composed of roughly equal amounts of collagens and GAGs, and rarely harbor leukocytes and blood vessels. In contrast, carcinoid valves have GAG-rich fibromyxoid tissue and contain a large number of leukocytes and vessels per square millimeter of tissue area (McDonald et al., 2002). Although blood vessels are generally absent from floppy valves, they were a prominent feature of anorexigen-exposed valves. However, even though more prominent as compared to floppy valves, anorexigen-exposed valves were still less vascular but more GAG-rich than carcinoid valves. Such features are indicative of a specific myxomatous process and of the existence of a distinctive pathologic process in anorexigen-exposed valves (McDonald et al., 2002). SMV has a strikingly similar morphology as well as composition to anorexigen-associated valvulopathy in humans. Valve-leaflets with SMV showed a greater valve thickness, a higher amount of GAGs and a lower amount of collagen (Elangbam et al., 2006).

In this study, we investigated whether 7-day 5HT subcutaneous injections led to structural and compositional abnormalities in conjunction with transcriptomic modulation (5HT2BR and 5HTT genes) in the aortic