Galectin-3 as a novel biotarget in cardiovascular alterations associated to development of severe aortic stenosis

La galectina-3, una nueva diana terapéutica para las alteraciones cardiovasculares asociadas al desarrollo de la estenosis aórtica severa

https://doi.org/10.23938/ASSN.0643

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ABSTRACT

Aortic stenosis is one of the most common heart valve diseases, as well as one of the most common causes of heart failure in the elderly. Currently, there are no medical therapies to prevent or slow the progression of the disease. When symptoms develop alongside severe aortic stenosis, there is a poor prognosis unless aortic valve replacement is performed. Aortic stenosis is a heterogeneous disease with a complex pathophysiology involving structural and biological changes of the valve, as well as adaptive and maladaptive compensatory changes in the myocardium and vasculature in response to chronic pressure overload. Galectin-3 serves important functions in numerous biological activities including cell growth, apoptosis, differentiation, inflammation and fibrosis. With evidence emerging to support the function of Galectin-3, the current review aims to summarize the latest literature regarding the potential of Galectin-3 as therapeutic target in aortic valve and cardiovascular alterations associated with aortic stenosis.

Keywords. Galectin-3. Aortic stenosis. Myocardial fibrosis. Valve calcification.

RESUMEN

La estenosis aórtica severa degenerativa (EA) es una enfermedad muy prevalente, cuya incidencia se incrementará en los próximos años debido al envejecimiento de la población. Actualmente no existe ningún tratamiento farmacológico que retarde su progresión y, cuando aparecen los síntomas, la cirugía de recambio valvular es la única opción. La EA se caracteriza por la calcificación de la válvula aórtica y por la aparición de fibrosis miocárdica. Sin embargo, no se conocen los mecanismos fisiopatológicos de la EA necesarios para identificar y desarrollar nuevas estrategias terapéuticas adecuadas. La Galectina-3 (Gal-3) regula funciones biológicas como el crecimiento, la diferenciación, la apoptosis, la inflamación o la fibrosis. Esta revisión resume los principales trabajos que describen el potencial de la Gal-3 como diana terapéutica para las alteraciones cardíacas y valvulares asociadas con el desarrollo de EA.

Palabras clave. Galectina-3. Estenosis aórtica. Fibrosis miocárdica. Calcificación valvular.

An. Sist. Sanit. Navar. 2019; 42 (2): 199-208

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Recepción: 30/04/2019
Aceptación provisional: 31/05/2019
Aceptación definitiva: 10/06/2019

AORTIC STENOSIS

Aortic stenosis (AS) is the most common heart valve disease (43%) and represents a major healthcare burden, since it is the third leading cause of cardiovascular disease[1]. Risk factors include male gender, smoking, diabetes mellitus, hypertension, high levels of circulating lipids, and metabolic syndrome[2]. With the increase in the aging population, there is a surge in the prevalence of calcific aortic valve disease. A prediction on the number of elderly (≥70 years) for the next few decades estimated that patients with severe AS will increase 2.4 fold by the year 2040 and more than triple by the year 2060[3]. Patients with AS have an 80% risk of valve replacement, progression to heart failure (HF), or death in the next 5 years after diagnosis[4].

The aortic valve is composed of three leaflets attached to the fibrous ring at the outlet of the left ventricle. The leaflets are composed of a dense extracellular matrix usually delineated into three layers with diff erent matrix composition, populated with valve interstitial cells (VICs) and the entire structure covered by valve endothelial cells: *lamina fibrosa* is the widest layer and faces the aortic or arterial side of the valve cusp, and it is composed principally by collagen circumferentially oriented to provide tensile strength[6]; *lamina spongiosa* is rich in glycosaminoglycans and proteoglycans that are believed to confer flexibility, dampen vibrations from closing, and resist delamination[7]; *lamina ventricularis* is a dense sheet of elastic fibres on the inflow side of the valve that is compliant, and provides elasticity and preload to the leaflets[8]. During embryogenesis the endothelial cells covering the primordial valve cushions migrate inside the underlying matrix and undergo endothelial to mesenchymal transition to become the interstitial cells[9].

The pathophysiology underlying calcific aortic valve disease remains incompletely defined and there are currently no effective medical treatments capable of altering its course[10]. Chronic inflammation, fibrosis and calcification play an important role in the progression of the disease [11]. The aortic valve leaflets are a highly specialized structure consisting mostly of VICs and complex extracellular matrix structures[12,13]. An inflammatory and fibrotic process in aortic valve in humans and animal models has been previously reported[14,15]. Aberrant remodelling of the extracellular matrix is also caused by the deregulated overexpression of matrix metalloproteinases, associated with inflammation[16]. These events occur during the activation of VICs towards an osteogenic-like phenotype, promoted by the up-regulation of bone morphogenetic proteins pathway[17]. Therefore, it has been shown that calcific aortic valve disease shares features with vascular calcification and atherosclerosis such as chronic inflammation, increased extracellular matrix remodelling, proliferation and differentiation of VICs and the development of calcific lesions[12,18]. Of note, although retrospective studies had suggested that statins could delay the hemodynamic progression rate of AS[19,20], in contrast, randomized controlled studies reported that a lipid-lowering strategy neither resulted in lower aortic valve-related events nor in a slower progression rate of stenosis[21,22].

Moreover, chronic pressure overload in AS induces a structural remodeling of the left ventricle and may promote HF[23] In the initial phases, the increased afterload imposed by aortic valve narrowing induces adaptive left ventricular hypertrophy that acts to maintain wall stress and cardiac output. Ultimately, this process decompensates, and patients transition from hypertrophy to HF and the development of symptoms and adverse cardiovascular events[18]. This transition is predominantly driven by myocardial fibrosis and myocyte cell death[24]. Thus, the transition from hypertrophy to HF plays a key role in AS. A better knowledge of the underlying mechanisms may highlight novel mediators of cardiac remodeling and decompensation which could identify biotargets for novel pharmacological therapies.

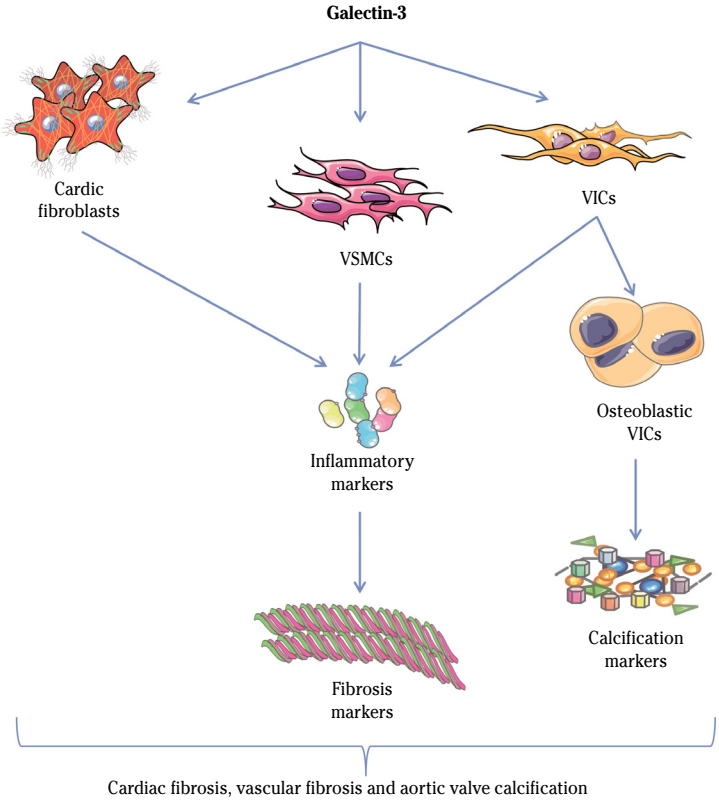
GALECTIN-3

Galectin-3 (Gal-3) is a 29–35 kDa protein, member of a β-galactoside binding lec-

tin family, localized in nucleus, cytoplasm, cell surface and extracellular space[25]. It is composed of a highly conserved N-terminal domain and a C-terminal carbohydrate recognition domain, which interacts with glycoproteins[26]. The damaging effects of Gal-3 have been associated to its capacity to bind matrix proteins such as cell surface receptors (integrins), collagen, elastin or fibronectin[27]. The expression of this lectin has been reported in many tissues, including heart, vessels and kidney[28]. Moreover, Gal-3 is expressed in many cell types of the cardiovascular system such as cardiac fibroblasts[29], vascular smooth muscle cells[30], endothelial cells[31], VICs[32] and inflammatory cells[33]. Gal-3 is involved in numerous physiological and pathological processes some of which, inflammation and fibrosis, are pivotal contributing to pathophysiological mechanisms in the development and progression of HF.

The effects of Gal-3 in cells from the cardiovascular system have been largely investigated (Fig.1).

Indeed, it has been demonstrated in cell culture that Gal-3 turns quiescent fibroblasts into myofibroblasts that produce and secrete matrix proteins, including collagen[29,34]. Gal-3 exerts its effects during several other stages of fibrogenesis besides collagen production, such as collagen maturation and cross-linking, which underscores the pivotal importance of Gal-3 in cardiovascular fibrosis[35,36]. Moreover, Gal-3 has emerged as a potential mediator of cardiovascular damage



VCMCs: vascular smooth muscle cells; VICs: valve interstitial cells.

Figure 1. Involvement of Galectin-3 in cellular pathophysiological processes associated with aortic stenosis.

in different pathological situations through its ability to stimulate key pro-inflammatory molecules[33]. Thus, it has been demonstrated in human cardiac fibroblasts that Gal-3 enhances the production and the secretion of proinflammatory and profibrotic mediators such as interleukin-1β, IL-6, monocyte chemoattractant protein-1, collagen type I, collagen type III, fibronectin as well as the activity of metalloproteinases-1, -2 and -9[37]. At the vascular level, Gal-3 increases the production and secretion of pro-fibrotic and pro-inflammatory markers in vascular smooth muscle cells[30], contributing to arterial stiffness. In endothelial cells, Gal-3 increases the expression of inflammatory factors (interleukin-6, interleukin-8, and interleukin-1β), chemokines (monocyte chemoattractant protein-1) and adhesion molecules[38]. Furthermore, Gal-3 modulates cell surface expression and activation of vascular endothelial growth factor receptor 2 in human endothelial cells contributing to the plasma membrane retention and exerting a pro-angiogenic function[39]. In VICs from aortic valves, Gal-3 also increases the secretion of pro-inflammatory and pro-fibrotic markers as well as the expression of calcification markers[32].

Beneficial effects of Galectin-3 blockade on aortic valve alterations in aortic stenosis

Chronic pressure overload due to AS results in pathological morphological changes in the cardiovascular system. These changes result in an initially compensatory phase, whose persistance could produce an important impact on cardiovascular function[40]. Pressure overload induces a modification in the aortic valves and the valve cusps become progressively thickened, fibrosed and calcified[41]. Moreover, a combination of endothelial damage and lipid deposition causes inflammation within the aortic valve that facilitates the infiltration of inflammatory cells which release proinflammatory factors[42,43,44]. In addition, matrix metalloproteinases secreted by VICs and inflammatory cells have an important and complex role in the restructuring of the aortic valve matrix[43]. Thus, abnormal remodeling in the aortic valve is also accompanied by the deregulated expression of metalloproteinases and inflammation[16]. As the stenosis-induced pressure overload progresses, wall shear stress across the aortic valve dramatically increases[45], activating transforming growth factor-β1[46], that can also induce fibrosis and calcification[44].

Gal-3 expression has been recently reported in VICs from aortic valves in patients undergoing aortic valve replacement[32]. Moreover, Gal-3 co-localized with the expression of osteogenic and inflammatory markers in human aortic valves[32]. Furthermore, *in vitro*, in human VICs, Gal-3 pharmacological inhibition with modified citrus pectin (MCP) as well as Gal-3 silencing attenuated the pro-inflammatory, pro-fibrotic and pro-osteogenic response[32]. A recent study described an association of Gal-3 with mortality after balloon aortic valvuloplasty, which is indicative of a contribution of local valvular Gal-3 expression to post-valvuloplasty restenosis[47]. In pressure overload, there is evidence of aberrant matrix deposition and valve fibrosis, which contributes to the calcification[48].

In agreement with these data, AS animals presented increased aortic valve inflammation, fibrosis, metalloproteinase activities and calcification markers. The pharmacological inhibition of Gal-3 was able to decrease the aortic valve inflammation, fibrosis, metalloproteinase activities and calcification in absence of increased blood pressure levels in the pressure overload group, showing the potential therapeutic benefit of Gal-3 inhibition both in the primary (i.e., in early stages of pressure overload) and secondary prevention settings (i.e., when pressure overload is installed) (Fig. 2)[49].

Beneficial effects of Galectin-3 blockade on cardiac alterations in aortic stenosis

AS accompanied by chronic pressure overload is a known precursor of left ventricular remodeling, involving cardiac fibrosis and inflammation. Patients display a marked variation in the magnitude of their

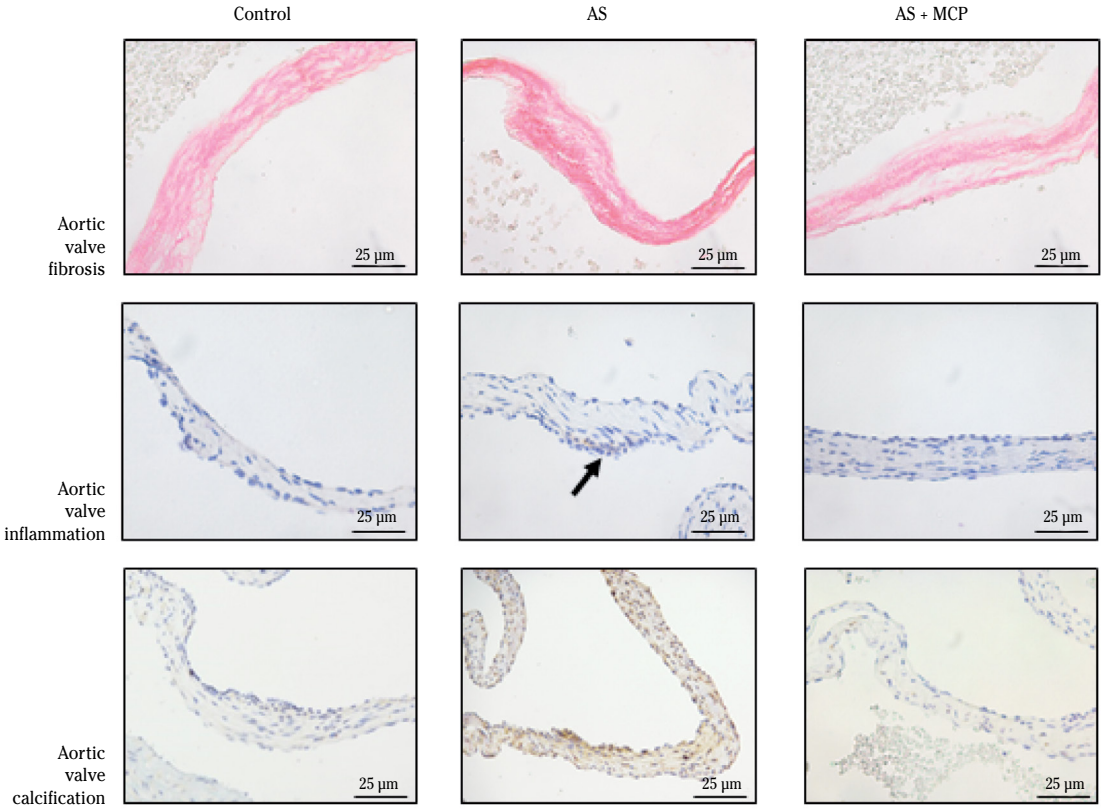


Figure 2. Beneficial effects of Galectin-3 blockade with modified citrus pectin (MCP) in aortic valve remodeling in an experimental model of aortic stenosis (AS). For collagen quantification (fibrosis), Sirius red staining was performed. Representative immunohistochemistry for cd68 and bone morphogenetic protein 4 are showed as examples of aortic valve inflammation and calcification respectively (from Ibarrola et al, 2017)[56].

left ventricular remodeling. This has recently been demonstrated to be of prognostic importance[50]. As with fibrosis in the valve, an imbalance in metalloproteinases and tissue inhibitor of metalloproteinase activities and inflammation have all been implicated in this process. In human myocardium, Gal-3 is mainly expressed by cardiac fibroblasts and can be found in extracellular matrix[51]. Moreover, increased Gal-3 expression has been previously shown in myocardium from AS patients with depressed ejection fraction, as compared to myocardium from AS patients with preserved ejection fraction[29], suggesting a role for Gal-3 in cardiac dysfunction associated with AS. Besides, cardiac Gal-3 expression has been found to be increased in animal models of pressure overload[34,51,52] and paralleled the severity of left ventricular diastolic dysfunction[52].

Several findings reported by our group deal with the potential consequences of Gal-3 overexpression in myocardium of AS patients. Firstly, cardiac Gal-3 overexpression is associated with cardiac fibrosis and inflammation[51]. Secondly, both cardiac and circulating Gal-3 levels positively correlated with cardiac fibrosis in AS patients[53]. Thirdly, a recent study showed that Gal-3 may serve as a prognostic biomarker after transcatheter aortic valve implantation by reflecting the degree of myocardial fibrosis[54]. Additionally, cardiac Gal-3 expression is associated with inflammatory markers

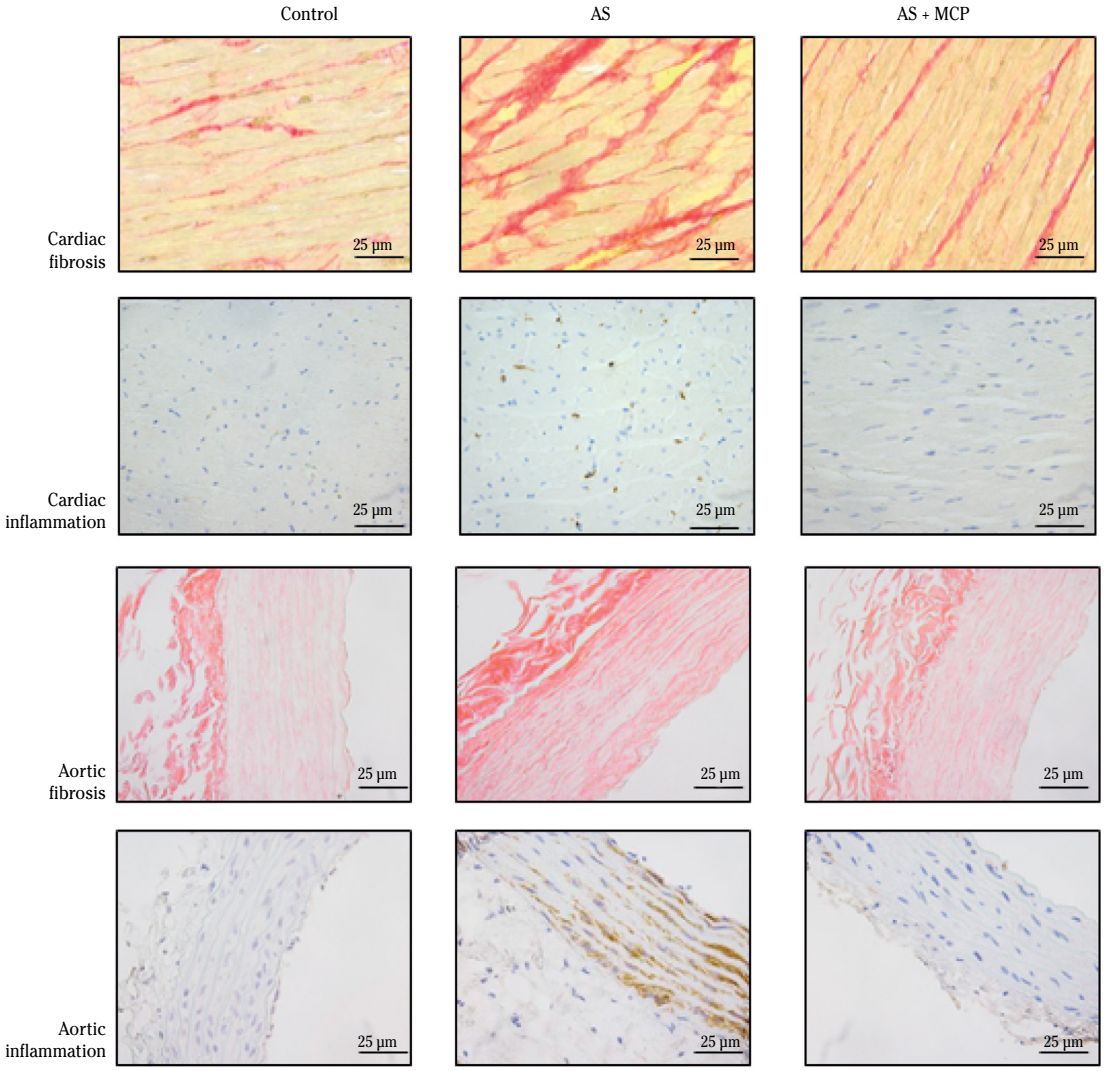


Figure 3. Beneficial effects of Galectin-3 blockade with modified citrus pectin (MCP) in cardiac and vascular remodeling in an experimental model of aortic stenosis (AS). For collagen quantification (fibrosis), Sirius red staining was performed in cardiac and aortic sections. In myocardium, cd68 was used as inflammatory marker, whereas in aortic sections monocyte chemoattractant protein-1 was chosen (from Ibarrola et al, 2017[56] and Arrieta et al, 2017[51]).

and metalloproteinase-1 in myocardial biopsies from AS patients[18], reinforcing the key role of this lectin in the inflammatory process and in extracellular matrix remodeling that accompanies the development of AS.

Previous studies have demonstrated that Gal-3 pharmacological inhibition prevented cardiac dysfunction, fibrosis and inflammation in several pathophysiological conditions such as hyperaldosteronism,[37,55] obesity[37] or hypertension[37]. Similar beneficial effects have been reported on cardiac fibrosis, remodeling and dysfunction in Gal-3 knockout mice subjected to thoracic aortic constriction[34]. In line with these find-

ings, pharmacological blockade of Gal-3 is able to prevent cardiac fibrosis, inflammation and functional alterations in an animal model of early stages of AS (Fig. 3). Thus, these results show the key role of Gal-3 in the cardiac remodeling associated with AS development and the beneficial effects of Gal-3 pharmacological inhibition on cardiac fibrosis and inflammation, the two key processes underlying the cardiac functional alterations which finally could affect cardiac function and AS progression, leading to HF.

Beneficial effects of Galectin-3 blockade on vascular alterations in aortic stenosis

At vascular level, pressure overload induces and increment of the aortic diameter and thickening of aortic wall through the extracellular matrix remodeling, characterized by an increment of fibrosis, inflammation and calcification in vessels and aortic valves[45,46]. Ascending aortic constriction is the most common surgical model for creating pressure overload-induced cardiovascular alterations. Gal-3 may contribute toward adverse cardiovascular effects in-part through an effect on aortic stiffness, effects which cannot be attributed to generalized inflammation.

In a recent study, it has been demonstrated that pharmacological Gal-3 inhibition by MCP could delay vascular remodeling and inflammation in a rat model of pressure overload (Fig. 3)[51,56]. Gal-3 inhibition exerts beneficial effects, decreasing aortic *tunica media* hypertrophy. Moreover, the use of MCP also decreases aortic fibrosis induced by pressure overload. Thus, the expression of collagen type I, fibronectin, α-smooth muscle actin, transforming growth factor-β1 and connective tissue growth factor was decreased in AS rats treated with the Gal-3 pharmacological inhibitor MCP. Complementarily, MCP treatment diminishes the expression of the inflammatory markers interleukin-6, interleukin-1β, tumor necrosis factor-α, monocyte chemoattractant protein-1, osteopontin, cd45 and cd68 in pressure-overloaded aortae (Fig. 3)[51,56]. These results suggest that Gal-3 may contribute toward adverse cardiovascular effects in part through an effect on aortic stiffness. In line with these findings, it has been shown that Gal-3 also contributes to ventricular-vascular uncoupling in HF patients[57].

CONCLUSIONS

Aortic stenosis is a disease of both the valve and the myocardium, characterized by fibrosis and calcification of valve leaflets, progressive left ventricular hypertrophy and cardiovascular fibrosis. In aortic stenosis, Gal-3 expression is increased in aortic valves, myocardium and aorta. Moreover, Gal-3 is colocalized with calcification markers in aortic valves, and with fibroblasts and extracellular matrix markers in myocardium. Gal-3 promotes inflammation, fibrosis and calcification in primary valvular interstitial cells and enhances the expression of fibrotic and inflammatory markers in cardiac fibroblasts and in vascular smooth muscle cells. Importantly, Gal-3 inhibition blocked aortic valve calcification, cardiac and vascular fibrosis and inflammation *in vivo* in an experimental model of pressure overload. Targeting Gal-3 may be an upstream therapeutic option for the treatment of aortic valve and cardiovascular remodeling that accompanies the progression of aortic stenosis. More in-depth mechanistic studies would be needed to understand the mechanisms by which Gal-3 inhibition blocks cardiovascular damage in aortic stenosis. Further clinical studies are required to establish the potential therapeutic benefit of Gal-3 inhibition in aortic stenosis patients.

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