

## Original Article

## Inhibition of Galectin-3 Pathway Prevents Isoproterenol-Induced Left Ventricular Dysfunction and Fibrosis in Mice

Giuseppe Vergaro, Mathilde Prud'homme, Loubina Fazal, Regine Merval, Claudio Passino, Michele Emdin, Jane-Lise Samuel, Alain Cohen Solal, Claude Delcayre

**Abstract**—Galectin-3 (Gal-3) is involved in inflammation, fibrogenesis, and cardiac remodeling. Previous evidence shows that Gal-3 interacts with aldosterone in promoting macrophage infiltration and vascular fibrosis and that Gal-3 genetic and pharmacological inhibition prevents remodeling in a pressure-overload animal model of heart failure. We aimed to explore the contribution of Gal-3 and aldosterone in mechanisms leading to heart failure in a murine model. Male mice with cardiac-specific hyperaldosteronism underwent isoproterenol subcutaneous injections, to be then randomized to receive placebo, a Gal-3 inhibitor (modified citrus pectin [MCP]), an aldosterone antagonist (potassium canrenoate), or MCP+canrenoate for 14 days. Isoproterenol induced a rapid and persistent decrease in left ventricular fractional shortening ( $-20\%$  at day 14); this was markedly improved by treatment with either MCP or canrenoate (both  $P < 0.001$  versus placebo). MCP and canrenoate also reduced cardiac hypertrophy and fibrosis and the expression of genes involved in fibrogenesis (*Coll-1* and *Coll-3*) and macrophage infiltration (*CD-68* and *MCP-1*). After isoproterenol, Gal-3 gene expression ( $P < 0.05$  versus placebo) and protein levels ( $-61\%$  and  $-69\%$  versus placebo) were decreased by both canrenoate and MCP. The combined use of antagonists of Gal-3 and aldosterone resulted in more pronounced effects on cardiac hypertrophy, inflammation, and fibrosis, when compared with either MCP or canrenoate alone. Inhibition of Gal-3 and aldosterone can reverse isoproterenol-induced left ventricular dysfunction, by reducing myocardial inflammation and fibrogenesis. Gal-3 likely participates in mechanisms of aldosterone-mediated myocardial damage in a heart failure murine model with cardiac hyperaldosteronism. Gal-3 inhibition may represent a new promising therapeutic option in heart failure. (*Hypertension*. 2016;67:00-00. DOI: 10.1161/HYPERTENSIONAHA.115.06161.)

• [Online Data Supplement](#)

**Key Words:** aldosterone ■ fibrosis ■ galectin-3 ■ heart ■ inflammation ■ isoproterenol

Galectin-3 (Gal-3) is a galactoside-binding lectin, which interacts with several extracellular matrix proteins, carbohydrates, and nonglycosylated proteins.<sup>1</sup> A correlation between Gal-3 expression and fibrosis has been found in several tissues: Gal-3 is upregulated in cases of liver, renal, and idiopathic pulmonary fibrosis.<sup>2,3</sup> Gal-3 is localized within the cytoplasmic space, but it can be translocated into the nucleus or secreted outside the plasma membrane via a nonclassical mechanism by several cell types.<sup>1</sup> In particular, macrophages can secrete Gal-3 in the extracellular space and activate resting fibroblasts into a matrix-producing phenotype.<sup>2</sup> Sharma et al<sup>4</sup> have shown that intrapericardial infusion of Gal-3 in healthy rats induces reduction in left ventricular (LV) ejection fraction and increase in collagen content. Furthermore, cardiac hypertrophy and dysfunction after angiotensin II infusion or transverse aortic constriction are blunted in Gal-3 knockout mice compared with wild-type, and Gal-3 pharmacological

inhibition by *N*-acetyllactosamine inhibits progression of LV remodeling in mice submitted to transverse aortic constriction.<sup>5</sup> Therefore, Gal-3 is likely involved in myocardial fibrogenesis in animal models of heart failure (HF) and participates in the mechanisms of cardiac remodeling.

The renin-angiotensin-aldosterone system plays as well a fundamental role in HF pathophysiology,<sup>6</sup> and the use of renin-angiotensin-aldosterone system antagonists represents a cornerstone in HF therapy.<sup>7</sup> Aldosterone infusion in rats with high-salt intake yields myocardial fibrosis via mineralocorticoid receptor (MR), which is preceded by macrophage infiltration and enhanced expression of inflammatory markers, such as osteopontin and monocyte chemoattractant protein-1.<sup>8</sup> Despite evidences on the role of oxidative stress and inflammation in the initiation of aldosterone-mediated cardiac fibrosis, the molecular machinery leading to end-organ damage remains largely unknown.<sup>9</sup>

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Although not conclusive, there is some experimental evidence that renin–angiotensin–aldosterone system and Gal-3 share signaling pathways in the development of cardiac fibrosis. In a model of double transgenic mice, with overexpression of both cardiac aldosterone synthase and the renin gene (AS-Ren mice), Azibani et al<sup>10</sup> showed that cardiac hyperaldosteronism in AS-Ren was associated with a massive macrophage infiltration, and with higher *Gal-3* expression and protein level, compared with Ren mice, Gal-3 being more abundant in the fibrotic areas. Recently, Calvier et al<sup>11</sup> observed an aldosterone-upregulated *Gal-3* expression in cultured vascular smooth muscle cells. They showed that administration of aldosterone and salt induced vascular hypertrophy, inflammation, fibrosis, and enhanced aortic *Gal-3* expression in hypertensive rats.<sup>11</sup> Noteworthy, these effects were antagonized by either MR antagonists or by modified citrus pectin (MCP), a water soluble indigestible polysaccharide binding to Gal-3 and blocking its activity through specific carbohydrate recognition domains.<sup>12,13</sup> Moreover, MCP has been demonstrated to prevent cardiac and renal damage when coadministered with aldosterone and salt in rats.<sup>14</sup>

Nonetheless, although a role of Gal-3 in the process of fibrogenesis and remodeling is clear, the evidence of its involvement in the aldosterone-mediated end-organ damage is still scarce in animal models of LV dysfunction and HF. The aim of this study was, therefore, to investigate the interaction of aldosterone and Gal-3 in pathophysiological mechanisms leading to HF in a murine model of LV systolic dysfunction.

## Methods

### Animals

Experimentations were conducted in agreement with the standards of animal care (National Institutes of Health Guide for the Care and Use of Laboratory Animals). Animal work was approved by the French National Committee of Ethic Reflexion on Animal Experimentation (CNREEA number, 09).

Transgenic mice with cardiac-specific overexpression of the aldosterone synthase gene (AS mice) were used for the experimental protocol. As previously reported, AS mice overexpress the AS gene in cardiomyocytes and have a 2-fold increased aldosterone concentration in the heart.<sup>15</sup> All mice were viable and were studied at 3 to 5 months.

### Induction of LV Systolic Dysfunction and Treatment

To induce LV systolic dysfunction, mice were treated with isoproterenol subcutaneous injections (300 mg/kg BID for 2 consecutive days). Echocardiography was performed again 1 day after the last injection. On the same day, AS mice were randomly assigned to receive treatment with either placebo (ISO group, n=12), the MR inhibitor potassium canrenoate (30 mg/kg per day as drinking water; ISO+C group, n=9), the Gal-3 pharmacological inhibitor MCP (100 mg/kg per day as drinking water; ISO+M group, n=10), or both canrenoate and MCP (ISO+C+M group, n=10). Echocardiography was performed again 7 and 14 days after the last isoproterenol injection. Mice were euthanized immediately thereafter. A group of mice injected with vehicle instead of isoproterenol and with no further pharmacological treatment served as control (control group, n=8).

### Echocardiography

Mice underwent baseline transthoracic echocardiography using a GE Vivid 7 machine (General Electric Company, Fairfield, CT) equipped with an 8- to 14-MHz linear transducer during light anesthesia with isoflurane (1%), as previously described.<sup>10</sup> Hearts were imaged in the 2-dimensional parasternal short-axis view, and 3 different frames

of an M-mode echocardiogram of the midventricle were recorded at the papillary muscle level. Each frame was analyzed to measure wall thickness and LV end-diastolic and LV end-systolic internal diameters. LV fractional shortening was calculated as (LV end-diastolic internal diameter–LV end-systolic internal diameter)/LV end-diastolic internal diameter and was taken as an index of LV systolic performance. All measurements were performed in a blinded fashion.

### Organ Weight and Tissue Analysis

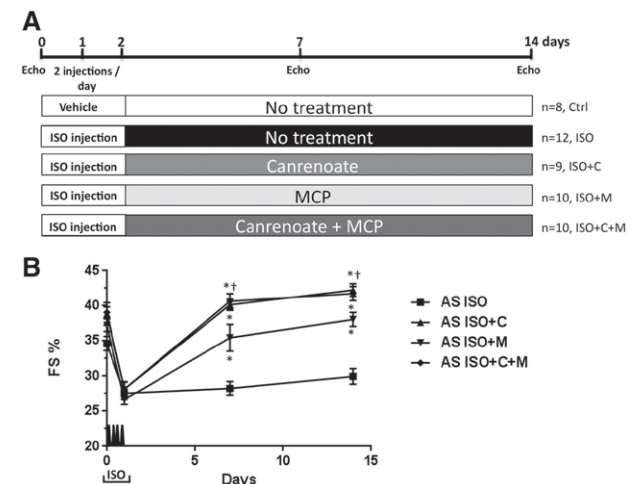
Mice were euthanized by an overdose of pentothal, and the hearts were arrested in diastole by KCl (30 mmol/L), quickly rinsed in cold saline, and blotted. Body weight, heart weight, weights of lung and liver, and tibia lengths were determined. The heart was transversally divided into 2 parts: the base part was embedded into Tissue-Tek O.C.T. (Sakura Finetek, Villeneuve d'Ascq, France) and frozen in liquid nitrogen for further histological and immunohistochemical analyses, and the apical part was snap frozen in liquid nitrogen. All samples were stored at –80°C until further analysis.

For measures of cardiac fibrosis, equatorial cryostat sections (7 μm) of the ventricles were stained with the collagen-specific picrosirius red stain (0.5% in saturated picric acid). Stained sections were observed under polarized light, and acquired images were used for the measure of collagen area/total ventricular surface ratio on at least 5 fields per sections, 3 sections per heart, and n>5 animals per groups. All images were acquired on a Leica microscope (Leica Microsystems, Rueil Malmaison, France) and recorded for further analysis. All the measures were performed in a blinded fashion using the IPLab software (BD Biosciences, San Jose, CA). Images were assembled using Photoshop (Adobe Systems, San Jose, CA).

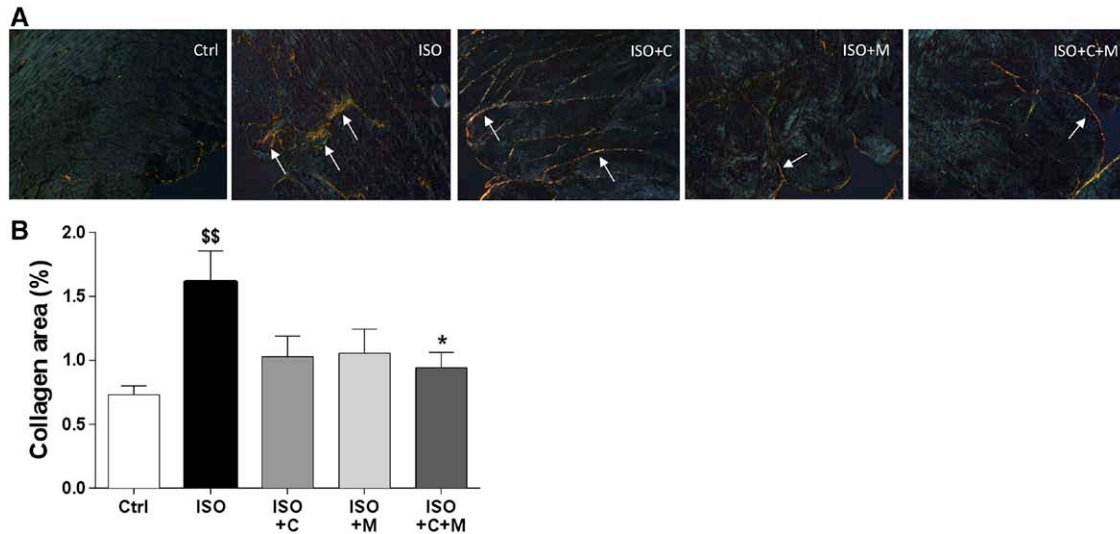
Western blot and immunofluorescence analyses were performed as published.<sup>10</sup>

### Gene Expression Analysis

For quantitative reverse transcription polymerase chain reaction analyses, total RNA extraction from the LVs, reverse transcription, and quantitative polymerase chain reaction were performed as previously described.<sup>10</sup> mRNA levels for genes of interest were normalized to the *GAPDH* mRNA levels and expressed as the relative change compared with the control samples.



**Figure 1.** Effects of aldosterone and galectin-3 antagonism on left ventricular systolic function. **A**, Schematic representation of the experimental protocol, including induction of left ventricular dysfunction and treatment regimens. **B**, Isoproterenol (ISO) induced a decrease in left ventricular fractional shortening (FS) 1 day after injections compared with baseline in all mice. Mice treated with canrenoate (ISO+C), modified citrus pectin (MCP; ISO+M), or both (ISO+C+M) showed a marked increase in FS compared with mice treated with ISO only (ISO) 1 week and 2 weeks after ISO injections; \* $P<0.001$  vs ISO and † $P<0.05$  vs ISO+M. Ctrl indicates control.



**Figure 2.** Galectin-3 and aldosterone antagonism reduces myocardial fibrosis. **A**, Sirius red staining in mice treated with placebo (control [Ctrl]), isoproterenol (ISO), ISO+either canrenoate (ISO+C) or modified citrus pectin (MCP; ISO+M), or both canrenoate and MCP (ISO+C+M) showing lower degree of myocardial fibrosis (arrows) in animals treated with aldosterone or galectin-3 antagonists. **B**, Computer-assisted evaluation showed complementary effects of canrenoate and MCP on the extent of fibrotic tissue; \* $P < 0.05$  vs ISO; \$\$ $P < 0.01$  vs Ctrl.

**Statistics**

Results are presented as mean±SEM, computed from the average measurements obtained from each group of animals. Differences among >2 experimental conditions were tested by the 1-way ANOVA test, followed by the Bonferroni test to analyze differences between groups. The Mann–Whitney *U*-test was used to assess statistical differences between 2 experimental conditions. Shapiro–Wilk test was used to assess normality. Statistical analyses were performed with IBM SPSS software, version 19.0.  $P < 0.05$  was considered significant.

**Results**

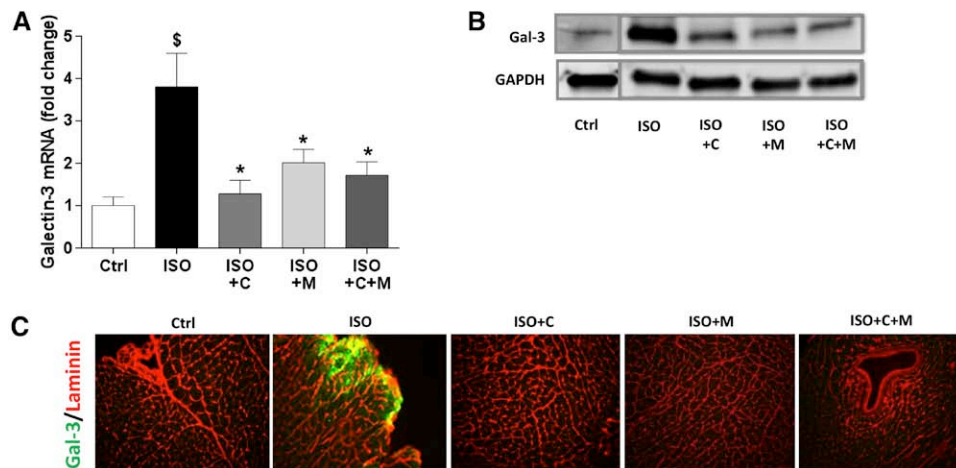
**Gal-3 and Aldosterone Inhibition Reverses LV Remodeling and Systolic Dysfunction**

The isoproterenol-treated AS mice (ISO group) showed a significant reduction in LV fractional shortening at day 1, which was maintained up to day 14 (Figure 1). Acute mortality after

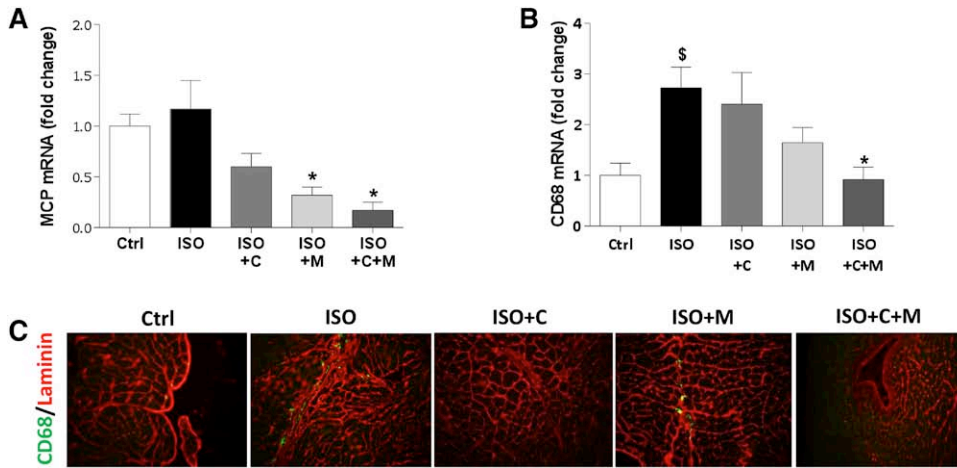
isoproterenol injection reached 10%. The ISO group showed increased LV end-systolic internal diameter and stable LV end-diastolic internal diameter after isoproterenol injections. The effects of isoproterenol were maintained up to 14 days. Treatment with either canrenoate or MCP (ISO+C and ISO+M groups, respectively) induced a decrease in LV end-systolic internal diameter and an increase in fractional shortening up to baseline values (Table S1 in the online-only Data Supplement). Similar results were observed when mice were treated with both canrenoate and MCP (ISO+C+M group; Figure 1; Table S1).

**Gal-3 and Aldosterone Inhibition Prevents Myocardial Hypertrophy and Interstitial Fibrosis**

At sacrifice, mice treated with either canrenoate or MCP showed less cardiac hypertrophy, as estimated by heart weight/



**Figure 3.** Galectin-3 and aldosterone pharmacological inhibition reduce cardiac galectin-3 expression and protein content. **A**, Treatment with canrenoate (ISO+C), modified citrus pectin (ISO+M), or both (ISO+C+M) decreased galectin-3 gene expression, estimated as mRNA content, compared with treatment with isoproterenol only (ISO). **B**, Similar results were obtained when galectin-3 protein content was evaluated by Western blot. **C**, Galectin-3 immunoblotting revealed significant accumulation in subendocardial areas (green) in ISO mice, which was abolished in ISO+C, ISO+M, and ISO+C+M groups; \* $P < 0.05$  vs ISO; \$\$ $P < 0.05$  vs control (Ctrl).



**Figure 4.** Effects of canrenoate and modified citrus pectin on inflammatory markers. **A** and **B**, A reduced expression of monocyte chemoattractant protein-1 (MCP-1) and CD68 was observed in mice treated with canrenoate (ISO+C) or modified citrus pectin (ISO+M) compared with mice treated with isoproterenol only (ISO); a more pronounced decrease was observed in the group treated with both canrenoate and MCP (ISO+C+M). **C**, CD68 immunolabeling revealed low accumulation (green areas) in ISO+M and even lower in ISO+C and ISO+C+M groups, compared with ISO; \* $P < 0.05$  vs ISO and \$ $P < 0.05$  vs control (Ctrl).

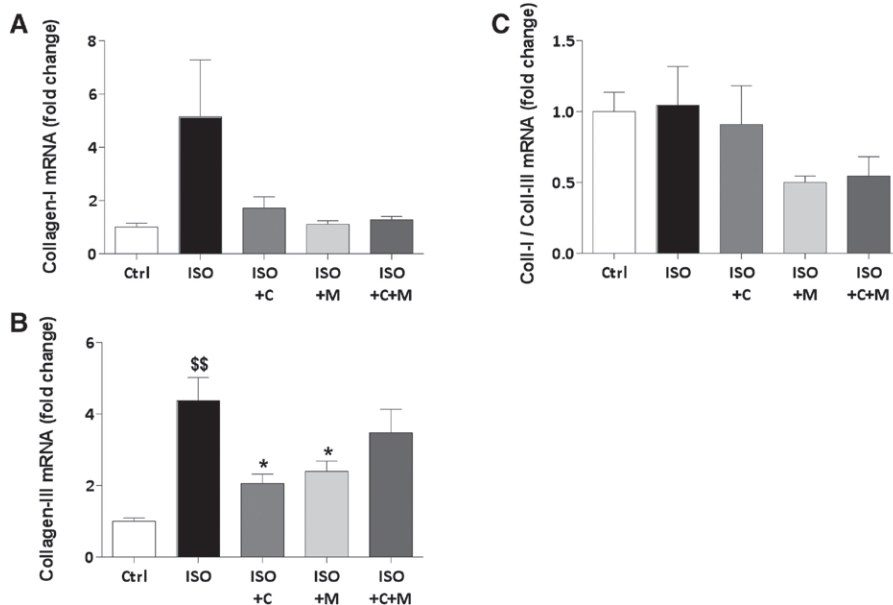
tibia length ratio. The ISO+C+M group exhibited even lower values of heart weight/tibia length compared with either ISO+C or ISO+M groups, thus suggesting a complementary effect of aldosterone and Gal-3 blockade in preventing myocardial hypertrophy (Table S2). Finally, all treatment groups showed lower organ congestion (as evaluated by lung and liver/tibia length ratios), with the ISO+C+M group demonstrating the lowest values of liver weight/tibia length ratio (Table S2).

As shown in Figure 2A and 2B, treatment with isoproterenol mainly resulted in the development of interstitial fibrosis (arrows). Treatment with canrenoate or MCP reduced the amount of fibrotic tissue in heart sections. The development of myocardial fibrosis was further blunted when mice were treated with Gal-3 antagonist in combination with MR

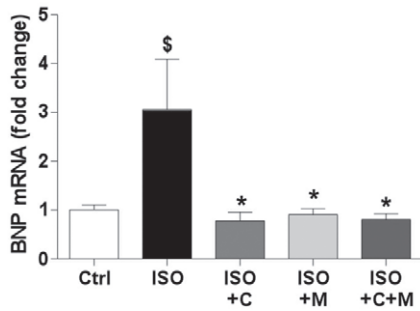
blocker, thus supporting a role for Gal-3 as a mediator of profibrotic pathways in LV dysfunction.

### Effects of Aldosterone and Gal-3 Inhibition on Myocardial Gal-3 Expression and Protein Content

To test the effects of different pharmacological approaches on myocardial Gal-3, cardiac *Gal-3* gene expression was evaluated by quantitative reverse transcription polymerase chain reaction (Figure 3A). In accordance with the myocardial inflammation and fibrogenesis elicited by high-dose isoproterenol,<sup>16</sup> ISO mice showed a markedly enhanced *Gal-3* expression. MR antagonism significantly reduced *Gal-3* expression (−64% versus ISO). Noteworthy, treatment with MCP alone or in combination with canrenoate was also associated with



**Figure 5.** Effects of canrenoate and modified citrus pectin on profibrotic markers. **A** and **B**, Treatment with canrenoate (ISO+C), modified citrus pectin (ISO+M), or their combination (ISO+C+M) induced a reduction in collagen 1 and collagen 3 gene expression, estimated as *Coll1* and *Coll3* mRNA content. **C**, A decrease in *Coll1/Coll3* ratio was also observed in treatment groups, being more pronounced in ISO+M and ISO+C+M mice; \* $P < 0.05$  vs ISO; \$\$ $P < 0.01$  vs control (Ctrl). ISO indicates isoproterenol.



**Figure 6.** Effects of canrenoate and modified citrus pectin on brain natriuretic peptide (BNP) expression. Treatment with canrenoate (ISO+C), modified citrus pectin (ISO+M), or their combination (ISO+C+M) induced a marked reduction in BNP gene expression, estimated as *Bnp* mRNA content; \* $P < 0.05$  vs ISO and \$ $P < 0.05$  vs control (Ctrl). ISO indicates isoproterenol.

decreased myocardial *Gal-3* expression (–47% and –55% versus ISO, respectively), raising the possibility that *Gal-3* may further stimulate—via a positive feedback—its own expression.

The *Gal-3* protein content in cardiac tissue was also assessed through Western blot analysis (Figure 3B), thus showing lower *Gal-3* in hearts from ISO+C, ISO+M, and ISO+C+M mice (–69%, –61%, and –76%, respectively) compared with ISO. Furthermore, immunolabeling (Figure 3C) revealed a significant accumulation of *Gal-3* in subendocardial areas (which are likely the most prone to the development of isoproterenol-induced damage) of ISO mice, whereas it was undetectable in samples from other treatment groups, thus supporting an inhibitory effect of canrenoate and MCP on *Gal-3* beyond the transcriptional level.

**Gal-3 and Aldosterone Inhibition Decreases Isoproterenol-Induced Inflammatory and Profibrotic Response**

Compared with hearts of the ISO group, mice treated with either canrenoate or MCP exhibited signs of a blunted

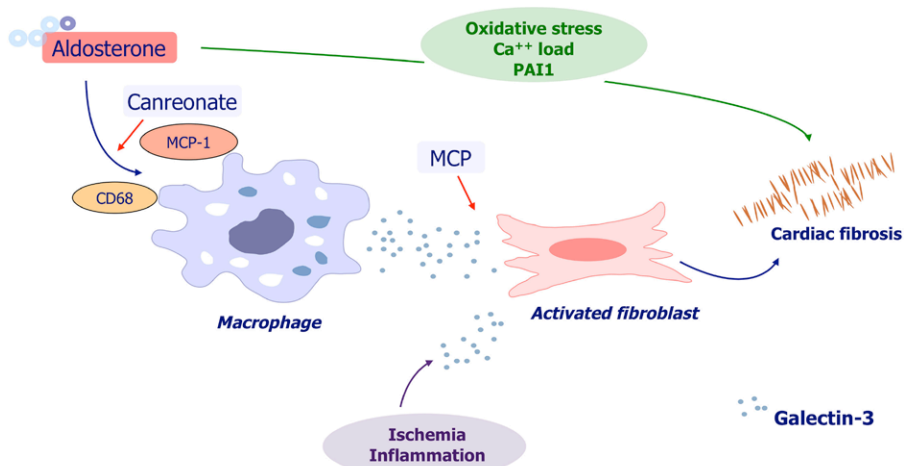
inflammatory response, as indicated by lower levels of *MCP-1* and *CD68* mRNAs (Figure 4A and 4B). The expression of these genes, both involved in macrophage recruitment and activation, was further decreased by combined treatment, suggesting a complementary effect of *Gal-3* antagonism over MR blockade. In line with results from reverse transcription polymerase chain reaction, indirect immunolabeling for CD-68 showed reduced positive cells in hearts from mice assigned to pharmacological treatment (ISO+C, ISO+M, and ISO+C+M) compared with ISO (Figure 4C).

We also tested whether the inhibitory effects of *Gal-3* and MR blockade on inflammatory response were associated with decreased fibrotic drive on myocardium through the analysis of the expression pattern of collagen I (*Coll 1*) and collagen III (*Coll 3*), 2 key structural components of the extracellular matrix. Expression of *Coll 1* was lower in hearts from mice treated with canrenoate, MCP, or their combination (–67%, –78%, and –75% versus ISO, respectively; Figure 5A). As concerns *Coll 3*, ISO+C and ISO+M mice showed a significant reduction in gene expression compared with ISO mice (–53% and –45%, respectively), whereas canrenoate+MCP exhibited only a modest decrease (–21%; Figure 5B). Because the relative increase of stiffer collagen phenotype (type I) to that of more elastic type (type III) is a feature of LV remodeling in dilated cardiomyopathy,<sup>17–19</sup> we finally evaluated the *Coll 1/Coll 3* expression ratio. Although the ratio was lower in ISO+C compared with ISO (–13%), treatment with MCP alone or together with canrenoate yielded a more pronounced reduction in *Coll 1/Coll 3* ratio (–52% and –48%, respectively; Figure 5C).

Interestingly, ISO+C, ISO+M, and ISO+C+M groups also showed a similar, marked decrease in brain natriuretic peptide gene expression (Figure 6).

**Discussion**

*Gal-3* and aldosterone antagonism reversed isoproterenol-induced LV systolic dysfunction and prevented the development of myocardial fibrosis in this mice model with selective



**Figure 7.** Putative model of aldosterone–galectin-3 (*Gal-3*) interplay in the development of myocardial fibrosis. Further to a downstream effect after aldosterone-mediated macrophage activation, *Gal-3* may be released as a consequence of different stimuli (including ischemia and inflammation). Similarly, aldosterone is likely to promote fibrosis through other *Gal-3*–independent mechanisms (such as oxidative stress, calcium overload, or increase in plasminogen activator inhibitor type 1 [PAI1]). Such alternative profibrotic pathways may explain the complementary effects observed on myocardial hypertrophy, inflammation, and fibrosis with combined aldosterone and *Gal-3* antagonism. CD68 indicates cluster of differentiation 68; MCP, modified citrus pectin; and MCP-1, monocyte chemoattractant protein-1.

cardiac hyperaldosteronism. Furthermore, combined Gal-3 and aldosterone blockade resulted in enhanced effects on cardiac inflammation and fibrosis, as well as on LV hypertrophy with respect to MCP or canrenoate alone.

Aldosterone has been largely studied as a mediator of cardiovascular remodeling in HF, and activation of MR is a crucial step in cellular and molecular machinery leading to myocardial inflammation, fibrosis, cardiomyocyte necrosis, and LV hypertrophy.<sup>20</sup> Nonetheless, despite this growing body of evidence, the exact mechanisms involved in aldosterone detrimental actions are still to be completely elucidated. More recently, Gal-3 has been reported to mediate inflammation and fibrosis and to hold a significant value in HF pathophysiology and prognostic stratification. The interaction between aldosterone and Gal-3 in the development of cardiovascular remodeling has been supported by recent experimental evidences from both animal and human studies. In particular, aldosterone has been demonstrated to induce Gal-3 secretion by macrophages through MR via the phosphoinositide 3-kinase inhibitor/Akt and nuclear factor- $\kappa$ B transcription signaling pathways.<sup>21</sup> In line with these findings, treatment with MR antagonists has been shown to downregulate cardiac expression of Gal-3 after experimental myocardial infarction.<sup>22</sup> Moreover, Calvier et al<sup>14</sup> have reported that cardiac and renal fibrosis is prevented by either pharmacological antagonisms (MCP) or genetic disruption of Gal-3 in rodents treated with aldosterone and salt, showing LV diastolic dysfunction.

Our findings support a role for Gal-3 in the development of fibrosis and dysfunction after cardiac injury. Furthermore, consistently with the presence of an interaction between aldosterone and Gal-3-mediated cardiac damage, we report a decrease in *Gal-3* gene expression and protein content in mice treated with the MR antagonist canrenoate.

High-dose isoproterenol, a sympathomimetic nonselective  $\beta$ -adrenergic receptor agonist, was used to induce LV dysfunction. Previous studies have demonstrated that isoproterenol produces diffuse, graded myocardial fibrosis in a patchy pattern, while maintaining a patent coronary vasculature,<sup>23,24</sup> likely subsequent to acute contraction band lesions.<sup>25</sup> Moreover, rodents submitted to isoproterenol subcutaneous injections show progressive LV remodeling, with impairment of both systolic and diastolic LV functions.<sup>24,26</sup> We, therefore, tested the effects of aldosterone and Gal-3 antagonism in an HF model associated with diffuse myocardial damage and cardiac hyperaldosteronism, in the absence of discrete myocardial infarction or afterload increase, as for the case of coronary artery ligation and transverse aortic constriction, respectively,<sup>25</sup> thus resembling clinical nonischemic dilated cardiomyopathy.

Treatment with MCP improved LV systolic function in AS mice after isoproterenol administration. This is the first demonstration that pharmacological inhibition of Gal-3 can reverse established systolic dysfunction and LV remodeling and further supports the potential clinical utility of targeting Gal-3 in HF therapy, through a tailored approach based on the evaluation of circulating levels of Gal-3.

Although the effects of aldosterone-induced macrophage activation and Gal-3 release are expected to be antagonized to the same extent by MCP, canrenoate, or their combination, our findings on the effects on cardiac hypertrophy and

myocardial inflammation and fibrosis with MCP+canrenoate administration are consistent with a complementary action of Gal-3 and MR blockade. Such observations may be explained by other Gal-3-independent mechanisms of fibrosis, known to be promoted by aldosterone, such as the induction of oxidative stress,<sup>9</sup> intracellular calcium accumulation,<sup>27</sup> or the increase in plasminogen activator inhibitor type 1.<sup>28</sup> Furthermore, cardiac expression and secretion of Gal-3 may be induced by other factors independent from MR activation, as ischemia and pro-inflammatory stimuli (Figure 7).<sup>29-31</sup>

## Perspectives

We demonstrate for the first time that pharmacological inhibition of Gal-3 can reverse isoproterenol-induced LV dysfunction in a murine model of HF with cardiac hyperaldosteronism, by reducing the development of myocardial inflammation and fibrogenesis in a complementary fashion over MR blockade. Our results only support an interaction between Gal-3 and aldosterone in the development of LV remodeling in HF, but further experiments will be needed to clarify the cellular and molecular basis underlying such interaction and to assess the potential utility of Gal-3 inhibition as a promising therapeutic option in HF, as an adjunct to conventional pharmacological neurohormonal antagonism, including the use of MR blockers.

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## Disclosures

None.

## References

1. Barondes SH, Cooper DN, Gitt MA, Leffler H. Galectins. Structure and function of a large family of animal lectins. *J Biol Chem*. 1994;269:20807-20810.
2. Henderson NC, Mackinnon AC, Farnworth SL, Poirier F, Russo FP, Iredale JP, Haslett C, Simpson KJ, Sethi T. Galectin-3 regulates myofibroblast activation and hepatic fibrosis. *Proc Natl Acad Sci U S A*. 2006;103:5060-5065. doi: 10.1073/pnas.0511167103.
3. Henderson NC, Mackinnon AC, Farnworth SL, Kipari T, Haslett C, Iredale JP, Liu FT, Hughes J, Sethi T. Galectin-3 expression and secretion links macrophages to the promotion of renal fibrosis. *Am J Pathol*. 2008;172:288-298. doi: 10.2353/ajpath.2008.070726.
4. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B, André S, Crijns HJ, Gabius HJ, Maessen J, Pinto YM. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation*. 2004;110:3121-3128. doi: 10.1161/01.CIR.0000147181.65298.4D.
5. Yu L, Ruirok WP, Meissner M, Bos EM, van Goor H, Sanjabi B, van der Harst P, Pitt B, Goldstein IJ, Koerts JA, van Veldhuisen DJ, Bank RA, van Gilst WH, Silljé HH, de Boer RA. Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis. *Circ Heart Fail*. 2013;6:107-117. doi: 10.1161/CIRCHEARTFAILURE.112.971168.
6. Patten RD, Konstam MA. Ventricular remodeling and the renin angiotensin aldosterone system. *Congest Heart Fail*. 2000;6:187-192.
7. McMurray JJ, Adamopoulos S, Anker SD, et al; Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology; ESC Committee for Practice Guidelines. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail*. 2012;14:803-869. doi: 10.1093/eurjhf/hfs105.

8. Sun Y, Zhang J, Lu L, Bedigian MP, Robinson AD, Weber KT. Tissue angiotensin II in the regulation of inflammatory and fibrogenic components of repair in the rat heart. *J Lab Clin Med*. 2004;143:41–51. doi: 10.1016/S0022214303001914.
9. Marney AM, Brown NJ. Aldosterone and end-organ damage. *Clin Sci (Lond)*. 2007;113:267–278. doi: 10.1042/CS20070123.
10. Azibani F, Benard L, Schlossarek S, Merval R, Tournoux F, Fazal L, Polidano E, Launay JM, Carrier L, Chaziantoniou C, Samuel JL, Delcayre C. Aldosterone inhibits antifibrotic factors in mouse hypertensive heart. *Hypertension*. 2012;59:1179–1187. doi: 10.1161/HYPERTENSIONAHA.111.190512.
11. Calvier L, Miana M, Reboul P, Cachofeiro V, Martinez-Martinez E, de Boer RA, Poirier F, Lacolley P, Zannad F, Rossignol P, López-Andrés N. Galectin-3 mediates aldosterone-induced vascular fibrosis. *Arterioscler Thromb Vasc Biol*. 2013;33:67–75. doi: 10.1161/ATVBAHA.112.300569.
12. Gunning AP, Bongaerts RJ, Morris VJ. Recognition of galactan components of pectin by galectin-3. *FASEB J*. 2009;23:415–424. doi: 10.1096/fj.08-106617.
13. Kolatsi-Joannou M, Price KL, Winyard PJ, Long DA. Modified citrus pectin reduces galectin-3 expression and disease severity in experimental acute kidney injury. *PLoS One*. 2011;6:e18683. doi: 10.1371/journal.pone.0018683.
14. Calvier L, Martinez-Martinez E, Miana M, Cachofeiro V, Rousseau E, Sádaba JR, Zannad F, Rossignol P, López-Andrés N. The impact of galectin-3 inhibition on aldosterone-induced cardiac and renal injuries. *JACC Heart Fail*. 2015;3:59–67. doi: 10.1016/j.jchf.2014.08.002.
15. Garnier A, Bendall JK, Fuchs S, Escoubet B, Rochais F, Hoerter J, Nehme J, Ambroisine ML, De Angelis N, Morineau G, d'Estienne P, Fischmeister R, Heymes C, Pinet F, Delcayre C. Cardiac specific increase in aldosterone production induces coronary dysfunction in aldosterone synthase-transgenic mice. *Circulation*. 2004;110:1819–1825. doi: 10.1161/01.CIR.0000142858.44680.27.
16. Liu L, Cui J, Yang Q, Jia C, Xiong M, Ning B, Du X, Wang P, Yu X, Li L, Wang W, Chen Y, Zhang T. Apocynin attenuates isoproterenol-induced myocardial injury and fibrogenesis. *Biochem Biophys Res Commun*. 2014;449:55–61. doi: 10.1016/j.bbrc.2014.04.157.
17. Thiedemann KU, Holubarsch C, Medugorac I, Jacob R. Connective tissue content and myocardial stiffness in pressure overload hypertrophy. A combined study of morphologic, morphometric, biochemical, and mechanical parameters. *Basic Res Cardiol*. 1983;78:140–155.
18. Mukherjee D, Sen S. Collagen phenotypes during development and regression of myocardial hypertrophy in spontaneously hypertensive rats. *Circ Res*. 1990;67:1474–1480.
19. Marijjanowski MM, Teeling P, Mann J, Becker AE. Dilated cardiomyopathy is associated with an increase in the type I/type III collagen ratio: a quantitative assessment. *J Am Coll Cardiol*. 1995;25:1263–1272. doi: 10.1016/0735-1097(94)00557-7.
20. Delcayre C, Swynghedauw B. Molecular mechanisms of myocardial remodeling. The role of aldosterone. *J Mol Cell Cardiol*. 2002;34:1577–1584.
21. Lin YH, Chou CH, Wu XM, Chang YY, Hung CS, Chen YH, Tzeng YL, Wu VC, Ho YL, Hsieh FJ, Wu KD; TAIPAI Study Group. Aldosterone induced galectin-3 secretion in vitro and in vivo: from cells to humans. *PLoS One*. 2014;9:e95254. doi: 10.1371/journal.pone.0095254.
22. Lax A, Sanchez-Mas J, Asensio-Lopez MC, Fernandez-Del Palacio MJ, Caballero L, Garrido IP, Pastor-Perez FJ, Januzzi JL, Pascual-Figal DA. Mineralocorticoid receptor antagonists modulate galectin-3 and interleukin-33/ST2 signaling in left ventricular systolic dysfunction after acute myocardial infarction. *JACC Heart Fail*. 2015;3:50–58. doi: 10.1016/j.jchf.2014.07.015.
23. Rona G, Chappel CI, Balazs T, Gaudry R. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. *AMA Arch Pathol*. 1959;67:443–455.
24. Teerlink JR, Pfeffer JM, Pfeffer MA. Progressive ventricular remodeling in response to diffuse isoproterenol-induced myocardial necrosis in rats. *Circ Res*. 1994;75:105–113.
25. Todd GL, Baroldi G, Pieper GM, Clayton FC, Eliot RS. Experimental catecholamine-induced myocardial necrosis. I. Morphology, quantification and regional distribution of acute contraction band lesions. *J Mol Cell Cardiol*. 1985;17:317–338.
26. Zhang W, Zhang J, Liu YK, Liu J, Wang X, Xu Q, Wang Y, Xu X, Dai G. Cardioprotective effects of oxymatrine on isoproterenol-induced heart failure via regulation of DDAH/ADMA metabolism pathway in rats. *Eur J Pharmacol*. 2014;745:29–35. doi: 10.1016/j.ejphar.2014.10.001.
27. Weber KT, Gerling IC, Kiani MF, Guntaka RV, Sun Y, Ahokas RA, Postlethwaite AE, Warrington KJ. Aldosteronism in heart failure: a pro-inflammatory/fibrogenic cardiac phenotype. Search for biomarkers and potential drug targets. *Curr Drug Targets*. 2003;4:505–516.
28. Huang W, Xu C, Kahng KW, Noble NA, Border WA, Huang Y. Aldosterone and TGF-beta1 synergistically increase PAI-1 and decrease matrix degradation in rat renal mesangial and fibroblast cells. *Am J Physiol Renal Physiol*. 2008;294:F1287–F1295. doi: 10.1152/ajprenal.00017.2008.
29. Hashmi S, Al-Salam S. Galectin-3 is expressed in the myocardium very early post-myocardial infarction. *Cardiovasc Pathol*. 2015;24:213–223. doi: 10.1016/j.carpath.2014.12.001.
30. Rubinstein N, Ibarregui JM, Toscano MA, Rabinovich GA. The role of galectins in the initiation, amplification and resolution of the inflammatory response. *Tissue Antigens*. 2004;64:1–12. doi: 10.1111/j.0001-2815.2004.00278.x.
31. Madrigal-Matute J, Lindholt JS, Fernandez-Garcia CE, Benito-Martin A, Burillo E, Zalba G, Beloqui O, Llamas-Granda P, Ortiz A, Egado J, Blanco-Colio LM, Martin-Ventura JL. Galectin-3, a biomarker linking oxidative stress and inflammation with the clinical outcomes of patients with atherothrombosis. *J Am Heart Assoc*. 2014;3:e000785. doi: 10.1161/JAHA.114.000785.

## Novelty and Significance

### What Is New?

- Galectin-3 and aldosterone interact in the development of left ventricular inflammation, fibrosis, and remodeling in a murine model of left ventricular dysfunction.
- Pharmacological inhibition of galectin-3 can reverse left ventricular systolic dysfunction after treatment with isoproterenol in mice.

### What Is Relevant?

- This study shows that galectin-3 is involved in the mechanisms of aldosterone-induced myocardial damage and supports a role for galectin-3 inhibition as a future therapeutic option in heart failure.

### Summary

Pharmacological inhibition of galectin-3 reduces the extent of myocardial inflammation and fibrosis in a complementary fashion over mineralocorticoid receptor blockade and reverses left ventricular dysfunction in a murine model of isoproterenol-induced heart failure.

**INHIBITION OF GALECTIN-3 PATHWAY PREVENTS ISOPROTERENOL-INDUCED LEFT VENTRICULAR  
DYSFUNCTION AND FIBROSIS IN MICE**

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**Short title:** Inhibition of galectin-3 in LV dysfunction



**Supplemental Table S1. Left ventricular diameters in mice.**

Left ventricular end-diastolic (LVEDD) and end-systolic (LVESD) diameters at baseline, and 1, 7 and 14 days (D1, D7 and D14, respectively) after the last isoproterenol injection in mice treated with isoproterenol only (ISO), or isoproterenol followed by canrenoate (ISO+C), modified citrus pectin (ISO+M) or canrenoate+modified citrus pectin (ISO+C+M); \* p<0.001 vs ISO, † p<0.05 vs ISO.

<b>LV diameters</b>	<b>Baseline</b>	<b>D1</b>	<b>D7</b>	<b>D14</b>
<b>ISO</b>				
<i>LVEDD (mm)</i>	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1
<i>LVESD (mm)</i>	2.7 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
<b>ISO+C</b>				
<i>LVEDD (mm)</i>	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.7 ± 0.1
<i>LVESD (mm)</i>	2.3 ± 0.1	2.8 ± 0.1	2.3 ± 0.0*	2.2 ± 0.1*
<b>ISO+M</b>				
<i>LVEDD (mm)</i>	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	3.9 ± 0.1
<i>LVESD (mm)</i>	2.6 ± 0.1	2.9 ± 0.1	2.6 ± 0.1†	2.4 ± 0.1*
<b>ISO+C+M</b>				
<i>LVEDD (mm)</i>	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	3.8 ± 0.0
<i>LVESD (mm)</i>	2.4 ± 0.1	2.8 ± 0.1	2.4 ± 0.1*	2.2 ± 0.0*

**Supplemental Table S2. Body and organ weight in mice.**

Whole body, and heart, lung and liver weights corrected for tibia length in mice treated with saline (Ctrl), isoproterenol (ISO), and isoproterenol plus canrenoate (ISO+C), modified citrus pectin (ISO+M) or canrenoate+modified citrus pectin (ISO+C+M); \* p<0.05 vs ISO.

<b>Variable</b>	<b>Ctrl</b>	<b>ISO</b>	<b>ISO+C</b>	<b>ISO+M</b>	<b>ISO+C+M</b>
Body weight (g)	27.6 ± 0.3	27.9 ± 0.8	26.4 ± 1.2	27.6 ± 1.2	26.8 ± 1.0
Heart weight/tibia length (mg/mm)	5.4 ± 0.1	6.3 ± 0.2	5.7 ± 0.2	5.7 ± 0.2	5.3 ± 0.1*
Lung weight/tibia length (mg/mm)	6.8 ± 0.4	7.8 ± 0.3	7.1 ± 0.3	7.2 ± 0.3	7.4 ± 0.3
Liver weight/tibia length (mg/mm)	80.0 ± 3.0	82.4 ± 1.8	79.0 ± 3.9	75.1 ± 3.6	69.6 ± 1.3*

## Inhibition of Galectin-3 Pathway Prevents Isoproterenol-Induced Left Ventricular Dysfunction and Fibrosis in Mice

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