ORIGINAL ARTICLE

Effect of Galectin-3 Deficit on Ventricular Remodeling After Coronary Occlusion in Mice

Efecto del déficit de galectina-3 sobre el remodelamiento ventricular post-oclusión coronaria en ratones

LUCIANA WILENSKY, NADIA L. MARTÍNEZ NAYA, PABLO CASSAGLIA, VERÓNICA VOLBERG[MTSAC], JULIA TAU, EUGENIA ARUANNO, ISAAC MORGUNOVSKY MICHELL, CELINA MORALES[MTSAC], GERMÁN E. GONZÁLEZ

ABSTRACT

Background: Galectin-3 (Gal-3) is a lectin that regulates the immune response. However, its role in remodeling and ventricular function after myocardial infarction (MI) is unknown.

Objective: The purpose of this study was to analyze whether Gal-3 deficit impairs remodeling and ventricular function after MI in mice.

Methods: Male Gal-3KO mice and their respective C57 controls underwent anterior descending coronary artery ligation or sham operation. Animals were then divided into four experimental groups: 1) C57 sham; 2) Gal-3 KO sham; 3) C57 MI and 4) Gal-3 KO MI. Seven days after surgery, an echocardiography was performed followed by euthanasia. Heart samples were collected to measure MI size and fibrosis using Masson's trichrome and picrosirius red, respectively, and assess macrophage infiltration and IL-6 expression.

Results: Left ventricular diameters were significantly increased in the C57 MI group compared with sham animals and the increase was even higher in the Gal-3 KO MI group. Moreover, ejection fraction decreased to 47%±2% in C57 MI and 37%±3% in Gal-3 KO MI mice ($p<0.02$), and infarct size increased from 39.4%±5% in C57 MI to 66.8%±5% in Gal-3 KO MI animals ($p=0.002$). Macrophage infiltration and fibrosis in the MI area were significantly reduced in Gal-3 KO MI mice ($p<0.001$ C57MI vs. Gal-3KO MI) without changes of IL-6 concentration in the left ventricular free wall (p=ns).

Conclusions: Gal-3 gene deletion is an important factor in repair kinetics, regulating macrophage infiltration and the degree of fibrosis in the infarct area, as well as early remodeling after MI.

Key words: Myocardial Infarction - Galectin-3 - Ventricular Remodeling

RESUMEN

Introducción: La galectina-3 (Gal-3) es una lectina que regula la respuesta inmune. Sin embargo, su rol en la remodelación y la función ventricular posinfarto de miocardio (IM) se desconoce.

Objetivo: Estudiar si el déficit de Gal-3 empeora la remodelación y la función ventricular pos-IM en ratones.

Material ymétodos: Se utilizaron ratones machos Gal-3 KO y su respectivo control C57 con ligadura de la coronaria descendente anterior o sham. Se conformaron cuatro grupos experimentales: C57 sham, Gal-3 KO sham, C57 IM y Gal-3 KO IM. A los 7 días poscirugía se les realizó ecocardiografía seguida de eutanasia y autopsia; se cuantificó el tamaño del IM y la fibrosis en cortes teñidos con tricrómico de Masson y picrosirius red, respectivamente, el infiltrado de macrófagos y la expresión de IL-6.

Resultados: Los diámetros del ventrículo izquierdo se incrementaron significativamente en el grupo C57 IM respecto del sham y dicho incremento fue aún mayor en el grupo Gal-3 KO IM. Además, la fracción de eyección disminuyó desde 47% ± 2% a 37% ± 3% en C57 IM y Gal-3 KO IM, respectivamente ($p< 0,02$). El tamaño del IM aumentó desde 39,4% ± 5% en los ratones C57 IM a 66,8% ± 5% en los animales Gal-3 KO ($p= 0,002$). El infiltrado de macrófagos y la fibrosis en el área del IM se redujeron en los ratones Gal-3 KO IM ($p< 0,001$ C57 IM vs. Gal-3 KO IM), mientras que la concentración de IL-6 en la pared libre del ventrículo izquierdo fue similar entre grupos (p=ns).

Conclusiones: La deleción de Gal-3 es un factor importante para la cinética del proceso reparativo regulando el infiltrado de macrófagos y el grado de fibrosis de la zona infartada, como también en la evolución temprana de la remodelación pos-IM.

Palabras clave: Infarto del miocardio - Galectina-3 - Remodelamiento ventricular

Rev Argent Cardiol 2015;83:287-291. http://dx.doi.org/10.7775/rac.v83.i4.5977

SEE RELATED ARTICLE: Rev Argent Cardiol 2014;83:282-284. http://dx.doi.org/10.7775/rac.v83.i4.6983

Received: 02/19/201 - *Accepted:* 04/15/2015

Address for reprints: Germán E. González - Departamento de Patología - Facultad de Medicina, Universidad de Buenos Aires - Pte. Uriburu 950, 2° Piso - CABA, Argentina - e-mail: gegonzal@fmed.uba.ar

Department of Pathology, School of Medicine, Universidad de Buenos Aires

[MTSAC] Full Member of the Argentine Society of Cardiology

Abbreviations

BW	Body weight	**LW**	Lung wet weight
EF	Ejection fraction	**MI**	Myocardial infarction
Gal-3	Galectin-3	**MT**	Masson´s trichrome
HF	Heart failure	**PSR**	Picrosirius red
HW	Heart weight	**SF**	Shortening fraction
IL6	Interleukin-6	**TL**	Tibial length
LVDD	Left ventricular diastolic diameter	**VR**	Ventricular remodeling
LVSD	Left ventricular systolic diameter		

INTRODUCTION

Myocardial infarction (MI) is the ischemic heart diseasewith highest morbidity and mortality and the most prevalent cause of heart failure (HF) worldwide, (1) including our country. (2). Myocardial infarction is a dynamic process, whose natural evolution comprises the repair of the infarct area by a fibrous scar and the onset of global ventricular remodeling (VR) that invariably modifies myocardial function and may lead to HF. (3)

Different lines of thought and research have postulated that a decrease in the repair process would reduce mortality and improve the progress of VR after MI. (4) However, other studies have shown that the decrease in the immune response and fibrosis generated in the MI area lead to a detrimental VR, characterized by greater dysfunction and ventricular dilatation. (5, 6) Previous studies from our laboratory showed that reduction of the inflammatory and fibrotic MI response, by inhibiting the renin-angiotensin system in rabbits submitted to experimental MI, had an unfavorable effect on VR. (6) However, few studies have assessed whether immunomodulation through the specific inhibition of innate and/or adaptive immunity modifies the evolution of MI and VR. (7) Recent studies have shown that galectin-3 (Gal-3), a lectin of approximately 30 kDa largely expressed in the immune system, (8) increases in HF patients and in experimental MI at 7-days post-MI. (9) Nevertheless, no studies have specifically evaluated whether Gal-3 gene deletion modifies the repair kinetics leading to greater adverse VR and myocardial dysfunction at 7 days post-MI. Therefore, the purpose of the present work was to study the effects of Gal-3 gene deletion on global VR and ventricular dysfunction post-MI, MI size, fibrosis and the repair kinetics. The results from these studies might help in the development of new therapeutic strategies to prevent adverse remodeling and the concomitant HF in the non-infarct region and regulate the repair of the MI area through the modulation of the inflammatory process.

METHODS

Male Gal-3 KO mice and their respective C57 controls (25-30 g body weight) were used. The animals were anesthetized with intraperitoneal ketamine/xylazine (75 mg/kg and 0.75 mg/kg, respectively) and following intubation with an endotracheal tube, they were ventilated with room air using a Harvard ventilator. After left lateral thoracotomy and pericardiotomy, the anterior descending artery was ligated with silk 7.0 (EthiconTM) or a sham procedure was performed. The thorax was closed and the animals were housed in individual cages in a quiet, air-conditioned environment for recovery and during the whole study period. Mice were divided into four experimental groups to study the effects of Gal-3 deficit on the infarct and non-infarct areas of the heart: 1) C57 sham (n=5); 2) Gal-3 KO sham (n=3); 3) C57 MI (n=13) and 4) gal3 KO MI (n=16).

Echocardiography ventricular function studies

Seven days after surgery, animals were anesthetized with tribromoetahnol (Avertin, 300 mg/kg, ip) immediately prior to euthanasia: Doppler echocardiography (Acuson Sequoia C256 ultrasound machine with 12 MHz transducer) was used to measure wall thickness and four ventricular dimensions in systole (LVSD) and diastole (LVDD), and ejection fraction (EF) and shortening fraction (SF) were calculated. (10)

Autopsy studies

Euthanasia was performed after the echocardiography study, and heart weight (HW), tibial length (TL) and lung wet weight (LW) were measured. A group of hearts was fixed in buffered formalin for further histological analysis.

Histological studies

Base-to-apex cross heart sections were embedded in paraffin and stained with hematoxilin-eosin, Masson´s trichrome (MT) and picrosirius red (PSR). Percent infarct size was quantified in sections stained with MT, (6) and fibrois in the infarct area in sections stained with PSR. (6)

Tissue cytokines

A group of hearts from each group was dissected to excise the MI area and quickly freeze it in liquid nitrogen for later processing and IL-6 assessment by ELISA.

Flow cytometry

Flow cytometry was used to quantify F4/F80+ (macrophages) cellular infiltration in the infarct area. Hearts were removed at autopsy and the MI area was rapidly excised. Then, mechanical and tissue enzymatic disaggregation was performed. The MI area was homogenized in PBS supplemented with bovine fetal serum at 4°C; then it was allowed to sediment and the homogenate was filtered through a nylon mesh. An aliquot of this cellular suspension was analyzed by flow cytometry to discard the presence of aggregates. To avoid interference in cytometry tests, red blood cells were lysed using cold ammonium chloride solution during 3 hours. The cellular suspension was subsequently centrifuged (15 min, 500 g, 4°C) and resuspended in PBS supplemented with fetal bovine serum. One hundred thousand cells in 10 ml final volume were

used for each test. Cells were incubated for 30 min at 4°C with normal mice serum to block Fc receptors and decrease the possibility of unspecific binding. Without washing or discarding the normal serum, 2.5 μl of monoclonal antibody labeled with rhodamine were added, and cells were incubated for another 30 minutes. Finally, cells were centrifuged and washed five times with cold PBS. They were then resuspended in 2% paraformaldehyde to preserve surface markers and cellular structure and were stored at 4°C in the dark until flow cytometry analysis. Anti-Ag80 and Ly-60 antibody labeling was used to identify macrophages and their subsets.

Statistical analysis

All values were expressed as mean±standard error of the mean. Analysis of variance (ANOVA) followed by the Bonferroni test was performed for the 4 groups, comparing differences between C57 sham vs. C57 MI, and C57 MI vs. Gal-3 KO MI. A p value<0.05 was considered as statistically significant.

Ethical considerations

All the experiments were approved by the Institutional Committee for the Care and Use of Animals (CICUAL)of the University of Buenos Aires and were performed according to valid guidelines on the care and use of laboratory animals.

RESULTS

During the first week, 32.0% of C57 MI and 44.4% of Gal-3KO MI animals died after permanent coronary artery ligation (p=ns). Mortality was mainly due to ventricular rupture, observed at autopsy by the presence of thoracic hemorrhage. Therefore, although mortality did not reach statistical significance, there was a clear trend to be higher in Gal-3 KO mice.

At 7 days post-MI, the hypertrophy index evaluated through the ratio between heart weight/body weight (HW/BW) (mg/g) was 4.9±0.1, 4.7±0.1 and 5.4±0.2 in the C57 sham, Gal-3 KO sham and C57 MI groups, respectively (p=ns), whereas it increased to 6.2±0.3 in Gal-3 KO MI (p<0.02 C57 sham and C57 MI vs. Gal-3 KO MI). Similarly, the degree of pulmonary congestion assessed by the LW/BW ratio (mg/g) was 5.9±0.3, 6.2±0.1 and 6.2±0.2 in C57 sham, Gal-3 KO sham and C57 MI, respectively (p=ns), but increased to 10.0±1.1 in the Gal-3 KO MI group (p<0.04 C57-sham and C57 MI vs. Gal-3 KO MI). These results suggest that Gal-3 gene deletion increases mortality, myocardial hypertrophy and pulmonary congestion in the acute phase of MI.

Infarct size was significantly increased in Gal-3 KO animals, from 39.4%±5% in C57 MI to 66.8%±5% in Gal-3 KO MI (p<0.002; C57 MI vs. Gal-3 KO MI; Figure 1). Moreover, in Gal-3 KO MI mice, fibrosis and macrophage infiltration in the MI area were significantly reduced from 29%±2% in C57 MI to 14%±2% in Gal-3 KO MI and from 14%±0.9% in C57 MI to 5%±0.1% in Gal-3 KO MI (p<0.001 C57 MI vs. Gal-3 KO MI; Figures 2 and 3). Surprisingly, the decrease of fibrosis and macrophage infiltration in the infarct area was independent from changes in IL-6 concentration (Figure 4). This suggests that in MI Gal-3 participates in the repair process regulating early macrophage infiltration and fibrosis in the MI area.

Gal-3 deficit was associated with increased adverse remodeling evidenced by enhanced ventricular dilatation. Echocardiography assessment showed that LVDD and LVSD of 4.4±0.1 mm and 3.5±0.1 mm in the C57 MI group increased to 4.8±0.2 mm and 4.1±0.2 mm in the Gal-3 KO MI group (LVDD: p<0.05 C57 MI vs. Gal-3 KO MI and LVSD: p<0.01 C57 MI and Gal-3 KO MI vs. C57 sham, and p<0.02 Gal-3 KO MI vs. C57 MI). These results were then confirmed during autopsy, where Gal-3 KO MI hearts were macroscopically more dilated compared with C57 MI hearts. At the same time, MI decreased EF from 71%±5% to 47%±2% and SF from 35%±4% to 20%±1% in C57 sham and C57 IM groups, respectively (p<0.001 C57 IM vs. C57 sham; Figure 5). This reduction was even greater in the Gal-3 KO MI group (37%±3% and 15%±2%, for EF and SF, respectively) (p=0.02 Gal-3 KO Mi vs. C57 MI, Figure 5).

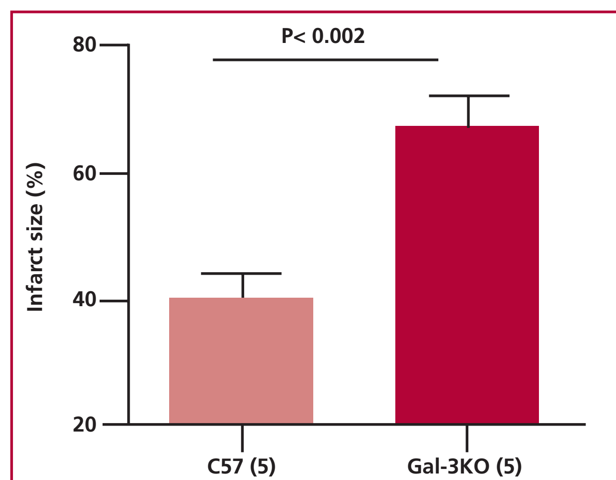

Fig. 1. Infarct size in C57 y Gal-3 KO animals, measured in histological sections stained with Masson´s trichrome.

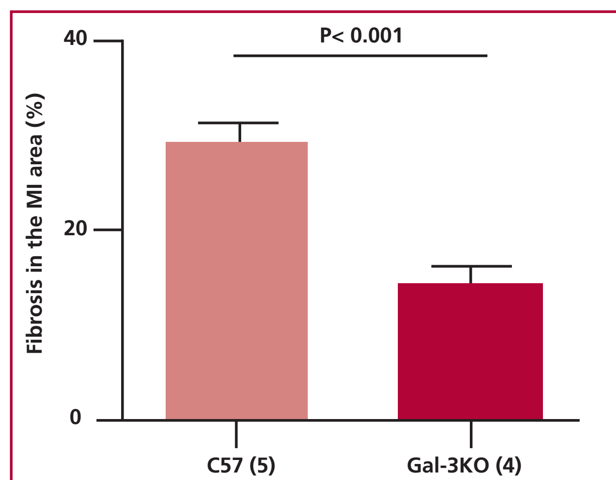

Fig. 2. Fibrosis in the infarct area at 7 days post-myocardial infarction in sections stained with picrosirius red.

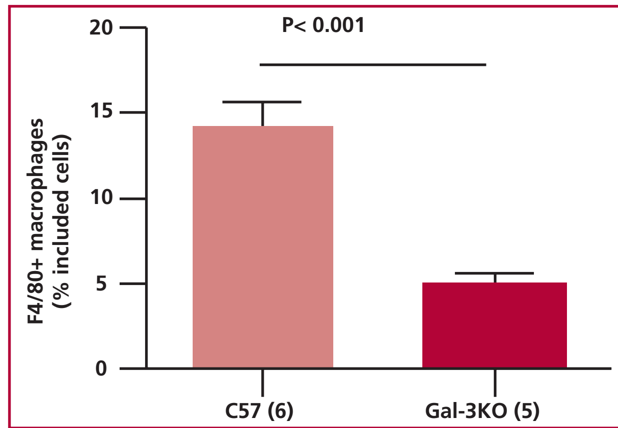

Fig. 3. Macrophage infiltration in the infarct area 7 days-post myocardial infarction measured by flow cytometry.

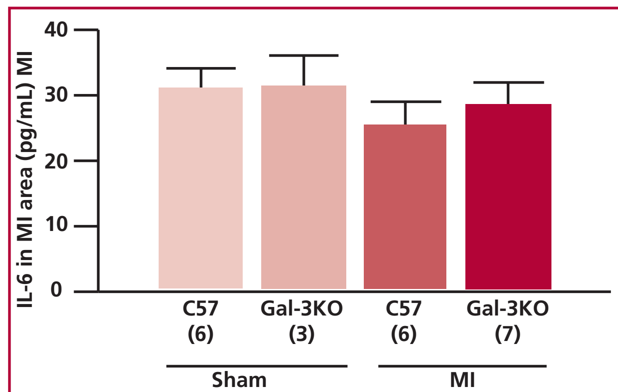

Fig. 4. IL-6 concentration assessed by ELISAin the infarct area.

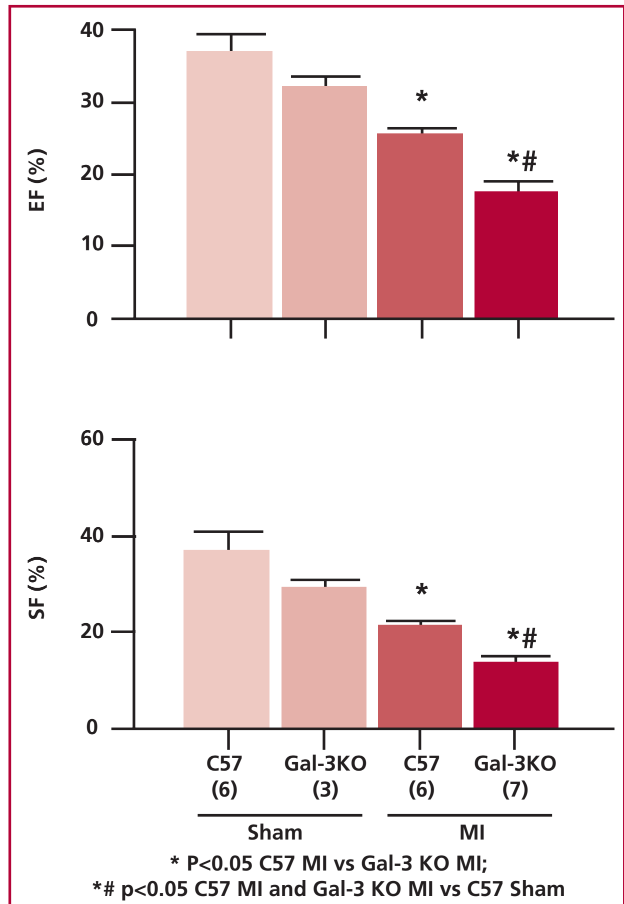

Fig. 5. Ventricular function assessed by echocardiography ejection fraction and shortening fraction.

These results indicate that Gal-3 gene deletion has an adverse effect not only on MI and early remodeling, but also on ventricular function at 7 days post-MI.

DISCUSSION

The present work shows for the first time that Gal-3 gene deletion increases MI size concomitantly with decreased macrophage infiltration and collagen deposit to generate the fibrotic scar. Interestingly, attenuation of the inflammatory and repair process observed in the MI area of Gal-3 KO animals occurred independently of changes in IL-6, a proinflammatory and profibrotic cytokine. This was accompanied by exacerbation of adverse VR and enhanced ventricular dysfunction, predisposing to the development of HF.

The present results reinforce the concept that the magnitude of the activated inflammatory response during MI area repair may be essential in the evolution of global structural changes. (11, 12) Cytokines, leukocytes and fibroblasts are the effectors of the repair process allowing the replacement of the necrotic myocardium by a scar. (13, 14)

The present work used an experimental model of MI in mice. In this sense, C57 and Gal-3 KO mice were submitted to permanent anterior descending coronary artery ligation during 7 days. Recent studies in rats with MI have shown that peak plasma Gal-3 levels are reached during this period. (15) These findings could imply that Gal-3 participates in the initial process of MI repair, regulating its kinetic progress through the magnitude of macrophage infiltration, the degree of fibrosis in the infarcted area and VR.

Different lines of research have shown that commonly used drugs for the treatment of HF reduce mortality and improve the outcome of remodeling after MI. These effects are primarily attributed to the decrease of ventricular hemodynamic load and then to the decrease of hypertrophy and fibrosis. (3) However, in previous works we showed that blockade of angiotensin II AT1 receptor since the beginning of MI reduces macrophage, lymphocyte and collagen infiltration in the MI area, delaying MI scarring. (6) Moreover, other studies evaluating the role of Gal-3 in the response to myocardial injury showed that in vivo angiotensin II infusion increased Gal-3 cardiac expression, suggesting that the proinflammatory effect of the former could be partially mediated by lectins. Myocardial Gal-3 has been shown to jointly increase active macrophage infiltration, fagocytic activity and collagen synthesis. (8) Another investigation demon-

strated that Gal-3 infusion in the pericardial sac had a proinflammatory effect, producing myocarditis, fibrosis and ventricular dysfunction. (7) Conversely, Gal-3 deficit was shown to reduce atherosclerotic inflammation. (16) It has been suggested that serum Gal-3 levels predict mortality in HF patients with preserved EF and that it would be a prognostic marker of HF even above the brain natriuretic peptide. (9)

The present study shows for the first time that Gal-3 deficit increases MI size and reduces macrophage infiltration and fibrosis in the injured area at 7 days post-MI. These results contradict previous findings of Li et al., (17) showing that Akt2 deletion, a serine/threonine kinase that physiologically stimulates (18) macrophage infiltration and Gal-3 expression in the myocardial injury area, was indirectly associated with larger MI size, suggesting that Gal-3 could favour inflammatory infiltration increasing MI size. The discrepancy between Li et al.'s and our study could be attributed to the ischemia-reperfusion model used in their case and the permanent coronary ligation used in ours.

We also showed that Gal-3 deficit in animals with MI increased ventricular dilatation and reduced systolic function. These effects might be explained, at least in part, by the increase in MI and reduction in macrophage infiltration and fibrosis in the MI area. The study could not discriminate to what degree each of these factors contributes to worsen adverse remodeling and dysfunction. However, as already mentioned, as each one constitutes a decisive factor in the progression of VR, their added contribution could explain the mechanism whereby Gal-3 deficit produced an unfavourable effect on VR and ventricular function. IL-6 concentration in the infarct area was similar between groups, suggesting that at least at one week post-MI Gal-3 effects on macrophage infiltration and the fibrosis repair process are independent of IL-6. These results are not surprising, as Kobara et al. recently found that IL-6 levels in the infarct area at 7 days post MI are similar to preischemic and remote area values. (19)

CONCLUSIONS

In conclusion, our data show for the first time that Gal-3 gene deletion has deleterious effects on the progress of post MI repair, which might accelerate the development of HF. Therefore, our results suggest that Gal-3 is an essential factor for the initiation and development of this process, VR and ventricular function, both in the MI area as in other non-infarct areas, and in addition they support previous findings reported by our group. (6) Further research on the intrinsic Gal-3-regulated mechanisms during the MI repair process and how they might contribute to global remodeling would allow to better understand their role in the pathophysiology and progression of HF.

Conflicts of interest

None declared

(See author´s conflicts of interest forms in the web / Supplementary Material)

REFERENCES

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoSMed 2006;3:e442. http://doi.org/ftr562

2. Estadísticas Vitales. Información Básica-Año 2010. Publicación del Ministerio de Salud de la Nación. 2011;5.

3. Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. Lancet 2006;367:356-67. http://doi.org/c4nzc5

4. Pfeffer MA, McMurray JJV, Velázquez EJ, Rouleau JL, Køber L, Maggioni AP, et al. Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. N Engl J Med 2003;349:1893-906. http://doi.org/bfj8sb

5. Ben Mordechai T, Holbova R, Landa-Rouben N, Harel-Adar T, Feinberg MS, Abd Elrahman I, et al. Macrophage subpopulations are essential for infarct repair with and without stem cell therapy. J Am Coll Cardiol 2013;62:1890-901. http://doi.org/f2qq33

6. González GE, Seropian IM, Krieger ML, Palleiro J, López Verrilli MA, Gironacci MM, et al. Effect of early versus late AT(1) receptor blockade with losartan on postmyocardial infarction ventricular remodeling in rabbits. Am J Physiol Heart Circ Physiol 2009;297:H375-86. http://doi.org/b5s4kn

7. Liu YH, D'Ambrosio M, Liao T-D, Peng H, Rhaleb NE, Sharma U, et al. N-acetyl-seryl-aspartyl-lysyl-proline prevents cardiac remodeling and dysfunction induced by galectin-3, a mammalian adhesion/growth-regulatory lectin. Am J Physiol Heart Circ Physiol 2009;296:H404-12. http://doi.org/dtk5jx

8. Sharma UC, Pokharel S, van Brakel TJ van Berlo JH, Cleutjens JP, Schroen B, et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. Circulation 2004;110:3121-8. http://doi.org/b5bbqs

9. de Boer RA, Lok DJ, Jaarsma T, van der Meer P, Voors AA, Hillege HL, et al. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. Ann Med 2011;43:60-8. http://doi.org/c9q7rc

10. Gao S, Ho D, Vatner DE. Echocardiography in mice. Curr Protoc Mouse Biol 2011;1:71-83. http://doi.org/dzjscg

11. Nahrendorf M, Pittet MJ, Swirski FK. Monocytes: Protagonists of infarct inflammation and repair after myocardial infarction. Circulation 2010;121:2437-45.http://doi.org/bpnhb7

12. Leuschner F, Panizzi P, Chico-Calero I, Lee WW, Ueno T, Cortez-Retamozo V, et al. Angiotensin-converting enzyme inhibition prevents the release of monocytes from their splenic reservoir in mice with myocardial infarction. Circ Res 2010;107:1364-73.http://doi.org/bbv7d2

13. Vandervelde S, van Amerongen MJ, Tio RA, Petersen AH, Van Luyn MJ, Harmsen MC. Increased inflammatory response and neovascularization in reperfused vs. non-reperfused murine myocardial infarction. Cardiovasc Pathol 2006;15:83-90. http://doi.org/cc667k

14. Morales C, Gonzalez GE, Rodriguez M, Bertolasi CA, Gelpi RJ. Histopathologic time course of myocardial infarct in rabbit hearts. Cardiovasc Pathol 2002;11:339-45. http://doi.org/bz2rz6

15. Sanchez-Mas J, Lax A, Asensio-Lopez MC, Fernandez-Del Palacio MJ, Caballero L, Garrido IP, et al. Galectin-3 expression in cardiac remodeling after myocardial infarction. Int J Cardiol 2014;172:e98-e101. http://doi.org/4w8

16. Nachtigal M, Ghaffar A, Mayer EP. Galectin-3 gene inactivation reduces atherosclerotic lesions and adventitial inflammation in ApoE-deficient mice. Am J Pathol 2008;172:247-55. http://doi.org/cb6496

17. Li X, Mikhalkova D, Gao E, Zhang J, Myers V, Zincarelli C, et al. Myocardial injury after ischemia-reperfusion in mice deficient in Akt2 is associated with increased cardiac macrophage density. Am J Physiol Heart Circ Physiol 2011;301:H1932-40. http://doi.org/cr842w

18. Arranz A, Doxaki C, Vergadi E, Martinez de la Torre Y, Vaporidi K, Lagoudaki ED, et al. Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization. Proc Natl Acad Sci USA 2012;109:9517-22. http://doi.org/4w9

19. Kobara M, Noda K, Kitamura M, Okamoto A, Shiraishi T, Toba H, et al. Antibody against interleukin-6 receptor attenuates left ventricular remodelling after myocardial infarction in mice. Cardiovasc Res 2010;87:424-30. http://doi.org/fcmh4h