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Letter to the Editor

Galectin-3 is essential for early wound healing and ventricular remodeling after myocardial infarction in mice ☆,☆☆

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Healing after myocardial infarction (MI) is a well-orchestrated time-dependent process that involves inflammation, tissue repair with progressive extracellular collagen matrix (ECM) deposition and scar formation. This scar should provide adequate tensile strength to prevent expansion, subsequent ventricular dilation [1], adverse ventricular remodeling (VR) and dysfunction [2]. Conversely, a defect in the healing process or an increase in the infarct size and parietal stress are major determinants to enhance the expansion and risk of adverse clinical outcomes, failure and death. Therefore, the idea to modify such an inflammatory process in order to revert the unfavorable course of VR post MI is still under debate [3,4]. Galectin-3 (Gal-3) is a β-galactoside-binding lectin widely expressed in the immune system. Classically, macrophages have been considered as the main source of Gal-3 [5]. Previous studies asserted that this lectin is up-regulated under different pathological conditions and stimulates inflammation and fibrosis [5,6]. Studies *in-vivo* showed that the infusion of Ang II increases the cardiac expression of Gal-3, suggesting that the pro-inflammatory effect of Ang II may be partially mediated by Gal-3. It has also been demonstrated that Gal-3 stimulates myocardial infiltration and phagocytic activity of active macrophages, and causes myocarditis, fibrosis and ventricular dysfunction [5,6]. During the last few years, plasma levels of Gal-3 were proposed as a strong prognostic marker of cardiac failure [7] and Mayr et al. recently showed that those levels correlated to the size of the MI in patients [8]. In rats with MI, Gal-3 reached its highest level of expression in the MI area after 7 days of evolution [9] suggesting that it could stimulate the post MI repair process. However, whether Gal-3 contributes or affects the well-orchestrated healing process after MI is not known. We hypothesize that the lack of Gal-3 prevents macrophage infiltration at the onset of the healing process, decreases the collagen deposition, and contributes to the adverse remodeling development after MI in mice. Accordingly, the aim was to study the role of the genetic deletion of Gal-3 on macrophage infiltration and fibrosis, as well as on infarct size, early remodeling and ventricular function in mice with permanent coronary ligature.

Adult male C57 and Gal-3 KO mice were subjected to permanent coronary artery ligature or Sham. Four experimental groups were performed: 1) C57 Sham; 2) Gal-3 KO Sham; 3) C57 with MI and 4) Gal-3 KO with MI. After 7 days echocardiography was performed in anesthetized animals (Avertin; 1.15 ml/kg). LV end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were measured, and the ejection fraction (EF) and the shortening fraction (SF) were calculated as well. Euthanasia was then performed and body, heart and lung weights (BW, HW and LW respectively) were recorded. The hearts were fixed in formalin, cut from apex to base into transversal slices and stained with Masson's Trichrome and Picrosirius Red to measure the MI size (%) and fibrosis (%) respectively. We also quantified F4/80+ macrophages in the MI area by flow cytometry. All experiments were approved by the Institutional Committee for Animal Care and Use of the University of Buenos Aires (CICUAL) and conducted in accordance with existing guidelines on the care and use of laboratory animals. During the first week, 32.0% of the C57 and 44.4% of the Gal-3 KO animals died after permanent ligature of the coronary artery (P = NS). Mortality was due mainly to ventricular rupture, observed during autopsy by the presence of thoracic hemorrhage. Thus, although

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mortality did not reach statistical difference, there was a clear tendency for mortality to be higher in Gal-3 KO mice. After 7 days of evolution, the HW/BW ratio (mg/g) was of 4.9 ± 0.1, 4.7 ± 0.1 and 5.4 ± 0.2 in the C57 Sham, Gal-3 KO Sham and C57 with MI groups respectively (P = NS), whereas this index was increased to 6.2 ± 0.3 in the Gal-3 KO MI (P < 0.02 C57 Sham and C57 MI *vs.* Gal-3 KO MI, respectively). Similarly, lung congestion as evaluated by LW/BW ratio (mg/g) was of 5.9 ± 0.3, 6.2 ± 0.1 and 6.2 ± 0.2 in C57 Sham, Gal-3 KO MI and C57 MI groups, respectively (P = NS), but it was 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). Taking these results all together suggest that genetic deletion of Gal-3 increases mortality, myocardial hypertrophy and pulmonary congestion at the acute stage of MI. The size of MI was significantly increased in Gal-3 KO animals (P < 0.002 C57-MI *vs.* Gal-3 KO-MI; Fig. 1A) but the genetic deletion of Gal-3 significantly reduced the macrophage infiltration and the fibrosis in the MI area (P < 0.001 C57 MI *vs.* Gal-3 KO MI; Fig. 1 B and C). This would suggest that Gal-3 participates in the MI initial stage repairing process regulating the macrophage infiltration and the fibrosis of the MI area. The lack of Gal-3 was also associated with increased adverse remodeling, evidenced by the increased ventricular dilation, evaluated by the LVEDD and the LVESD that were 4.4 ± 0.1 mm and 3.5 ± 0.1 mm in the C57-MI group and they increased to 4.8 ± 0.2 mm and 4.1 ± 0.2 mm in the Gal-3 KO MI group (LVEDD: P < 0.05 Gal-3 KO MI *vs.* C57-MI and; LVESD: P < 0.01 C57 MI and Gal-3 KO MI *vs.* C57 Sham and, P < 0.02 Gal-3 KO MI *vs.* C57 MI; Fig. 2). These results were subsequently verified during autopsy, where it was confirmed that the hearts of the Gal-3 KO animals with MI were macroscopically more dilated in comparison to those of the C57 MI hearts. At the same time, the MI produced a decrease of the EF from 71 ± 5% to 47 ± 2% and of the SF from 35 ± 4% to 20 ± 1% in the C57 Sham and C57-MI groups, respectively (P < 0.001 C57 MI *vs.* C57 Sham; Fig. 2). This reduction was even higher 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; Fig. 2). These results provide evidence that the genetic deletion of Gal-3, produces an adverse effect not only on MI and early remodeling, but also on the ventricular function at 7 days post-MI. Summarizing, our data shows for the first time, that the genetic deletion of Gal-3 increases the MI size, and prevents collagen deposition and macrophage infiltration in the MI area. It also aggravates the adverse remodeling and ventricular dysfunction, consequently accelerating the development of cardiac failure. Thus, our results strongly suggest that Gal-3 is an essential factor for the onset and development of the healing and fibrogenic process of the MI area, as well as the remodeling and the ventricular function in the first week of MI evolution. These results emphasize the previous findings of our group showing that an intervention which attenuates the initial healing process in MI could be counterproductive for the natural evolution of VR [10]. New experimental studies on the intrinsic mechanisms regulated by Gal-3 during the healing process of the MI, and how these could contribute to the

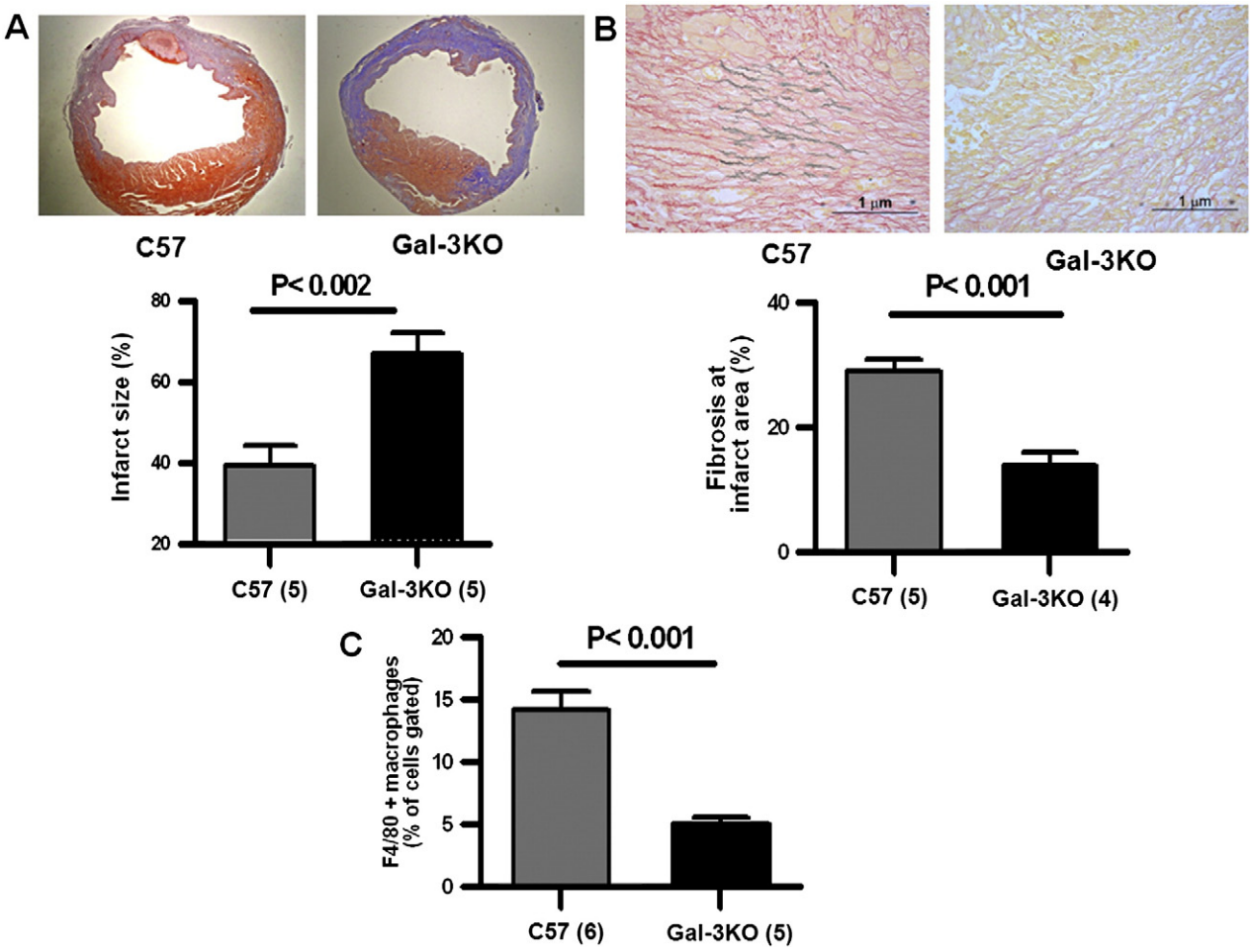

Fig. 1. A: MI size quantification by planimetry after 7 days post ligation of the left coronary artery. The deficit of Gal-3 increased the MI size. At the upper left panel, 2 representative images of a midventricular section, stained with Masson's Trichrome (20×) are shown, where we can clearly appreciate a greater infarct expansion, thinning and ventricular dilation. B: Quantification of fibrosis in the sections stained with Picrosirius Red, corresponding to the MI area (upper right panel; 200×); the Gal-3 deficit significantly reduced the fibrosis at the infarct zone after 7 days of evolution. C: Quantification of F4/80+ macrophages by flow cytometry. The Gal-3 deficit reduced the macrophage infiltration in the MI area after 7 days of evolution. Number of animals between brackets.

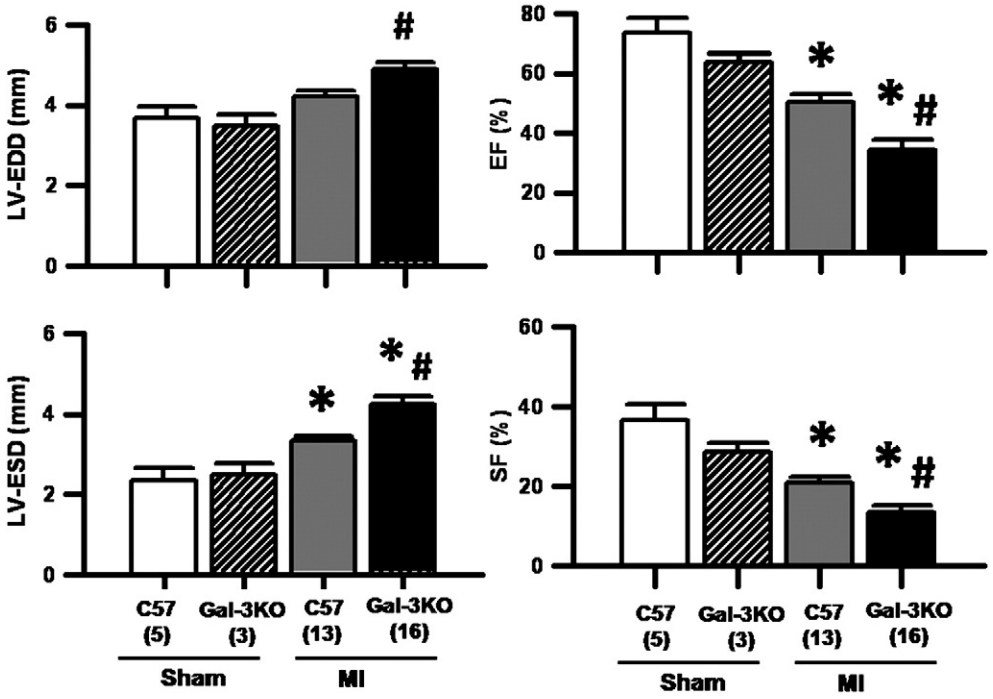

Fig. 2. Evaluation of remodeling and ventricular function by echocardiography after 7 days post-MI. In mice with MI, the deficit of Gal-3 increased the ventricular dilation, evaluated by the end diastolic diameter (LVEDD, left upper panel) and the end systolic diameter (LVESD, left lower panel), respectively. The aforementioned dilation was accompanied by a decreased ejection fraction (EF, right upper panel) and the shortening fraction (SF: right lower panel). *: $P < 0.01$ C57 MI and Gal-3 KO MI *vs.* C57 Sham; #: $P < 0.05$ C57 MI *vs.* Gal-3 KO MI. Number of animals between brackets.

global remodeling, would allow us to comprehend in greater detail the role of Gal-3 in MI pathophysiology and the progression to CF.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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