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Letter to the Editor

Galectin-3 expression in cardiac remodeling after myocardial infarction

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Acute myocardial infarction (MI) is a major cause of chronic heart failure (CHF) [1]. After MI, inflammatory and fibrotic responses take place in both infarcted and non-infarcted myocardium; both contribute significantly to adverse cardiac remodeling and ventricular dysfunction [2]. The complex biology of remodeling remains incompletely characterized. Therefore, the characterization of novel mediators of myocardial remodeling and the timing of their action are essential for the development of new cardioprotective and reparative strategies aimed to prevent fibrosis after MI and to slow the progression from MI to overt CHF.

Higher levels of circulating galectin-3 (Gal-3) have been associated with increased risk for incident HF, adverse left ventricular (LV) remodeling, decompensated HF and worse prognosis [3–6]. Besides being an emerging biomarker in CHF, Gal-3 has been suggested to play an important role in the pathophysiology of LV remodeling and CHF [7]. However, no data exist about the expression of Gal-3 in cardiac tissue after MI. The present study aimed to study the expression of Gal-3 in cardiac tissue after MI, the kinetics of Gal-3 in infarcted and non-infarcted regions, and the association between Gal-3 and fibrosis in the process of cardiac remodeling.

Fifty male Wistar rats, weighing 220–300 g, underwent permanent left anterior descending coronary artery ligation and were randomly assigned to five groups (10 rats per group), according to the time assigned from surgery to the sacrifice: 1-week, 2-weeks, 4-weeks, 3-months and 6-months. The MI size evaluated by echocardiography 24 h post-MI was similar among the infarcted groups (21.3 ± 14.6; $p = 0.318$). MI size was calculated as the percentage of akinetic segments using a representation system of 16 segments, obtained from three right parasternal short-axis views at the level of mitral valve, papillary muscles and apex. Eight sham operated animals without ligation were also analyzed as the control group. As expected, the presence of MI was associated with a significant drop of LV ejection fraction (LVEF: 50.1 ± 9.8 vs. 40.2 ± 14.8%, sham vs. MI, $p < 0.05$) and fractional area change (FAC: 50.1 ± 9.8 vs. 38.9 ± 10.1%, sham vs. MI, $p < 0.05$), while the LV end-diastolic and end-systolic volumes were significantly increased during the follow-up in relation with the LV remodeling after MI (LVEDV: 0.94 ± 0.3 vs. 1.44 ± 0.3 ml/kg, sham vs. MI, $p < 0.01$; LVESV: 0.5 ± 0.1 vs. 0.8 ± 0.3, ml/kg, sham vs. MI, $p < 0.01$). Macroscopically, the hearts of MI rats were larger and more spherical compared to those of sham rats. The myocardial scar was evident from 1 week post-MI and completely formed from 2 weeks. In addition MI rats showed, as compared with sham rats, an increased ratio of heart/body weight (2.4 ± 0.08 vs. 2.8 ± 0.06 mg/g, sham vs. MI, $p = 0.01$) and lungs/body weight (4.4 ± 0.2 vs. 5.4 ± 0.2 mg/g, sham vs. MI, $p = 0.006$).

Cardiac expression of several markers was assessed in both infarcted and non-infarcted myocardium. The mRNA expression of Gal-3 and fibrosis markers was analyzed by quantitative RT-PCR, while the protein expression of Gal-3 and CD68 (marker of macrophage presence) was analyzed by immunohistochemical staining. In the infarcted myocardium, Gal-3 mRNA expression was increased and showed a maximum level at 1 week post-MI (96.1 ± 25.4 fold of control), with a progressive decrease in the next weeks (Fig. 1A). The kinetics of mRNA expression of collagen I (Col I), collagen III (Col III) and TIMP-1 in the infarcted area showed similar behavior than that of Gal-3, with a peak at 1 week post-MI (Fig. 1B–D). In the infarcted area, both Gal-3 and CD68 protein expression showed a high peak at 1 week post-MI, and a progressive decrease in the next weeks (Fig. 1E).

In the non-infarcted area, Gal-3 mRNA expression levels were significantly increased in the MI rats compared with sham group; this increase was lower than that observed in the infarcted area, and showed a late maximum at 6 months post-MI (3.1 ± 0.53 fold of control) (Fig. 2A). The increase of mRNA expression of TIMP-1 reached significance as compared with sham group since 1 week post-MI (Fig. 2B). Although the mRNA levels of both Col I and Col III

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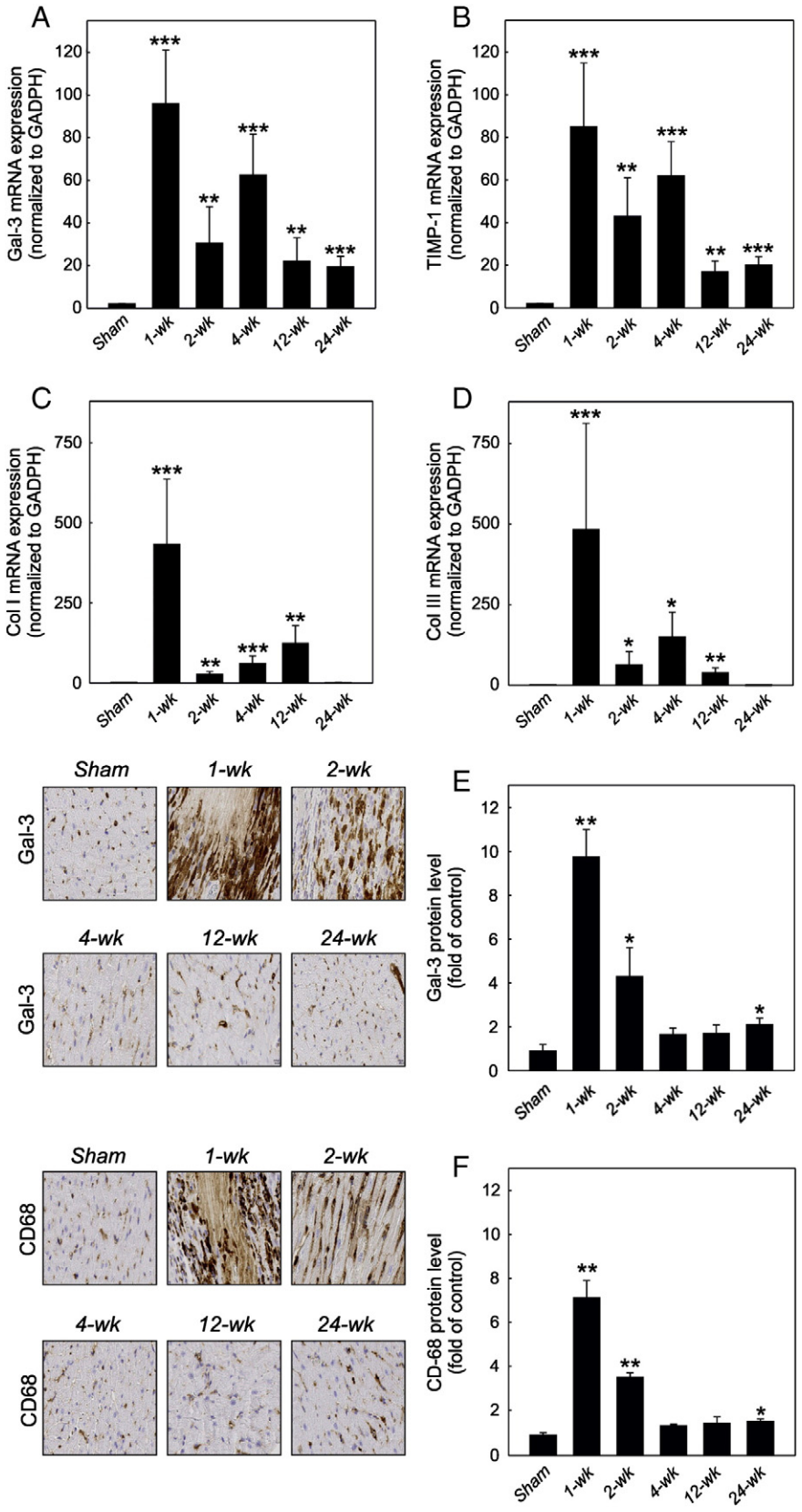

Fig. 1. Kinetics of expression of Gal-3, CD-68 and fibrosis markers in the infarcted myocardium. Quantitative RT-PCR analysis of mRNA expression of Gal-3 (A), TIMP-1 (B), Col I (C) and Col III (D). Representative photomicrographs (40×) illustrating the immunohistochemical staining of Gal-3 (E) or CD68 (F) in the absence of MI (sham) or in the infarcted myocardium from MI rats. The analysis of Gal-3 or CD68 expression in the infarcted myocardium is also showed in panels E and F, respectively. Data are expressed as fold of sham group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to sham group.

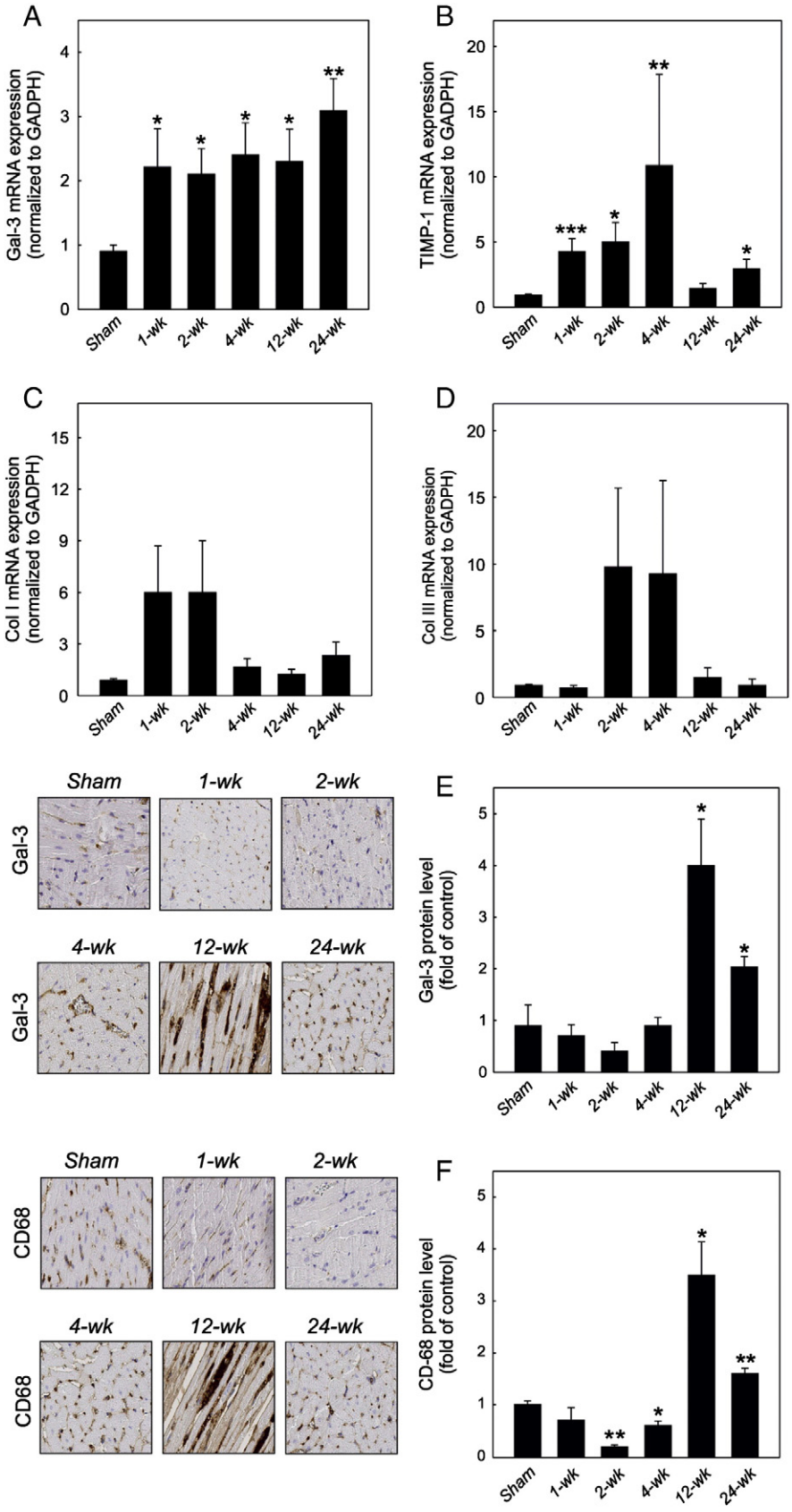

Fig. 2. Kinetics of expression of Gal-3, CD-68 and fibrosis markers in the non-infarcted myocardium. Quantitative RT-PCR analysis of mRNA expression of Gal-3 (A), TIMP-1 (B), Col I (C) and Col III (D). Representative photomicrographs (40×) illustrating the immunohistochemical staining of Gal-3 (E) or CD68 (F) in the absence of MI (sham) or in the non-infarcted myocardium from MI rats. The analysis of Gal-3 or CD68 expression in the non-infarcted myocardium is also showed in panels E and F, respectively. Data are expressed as fold of sham group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to sham group.

showed an increase at 2 weeks post-MI, these increases were not significant compared to sham group (Fig. 2C and D). In the non-infarcted area, Gal-3 protein levels were similar to sham group in the first 4 weeks, but it showed a late peak at 3 months (Fig. 2E). The protein expression of CD68 showed a similar kinetic than Gal-3, with a later peak at 3 months post-MI (Fig. 2F).

The correlation analysis did not show any correlation between Gal-3 in the infarcted area and in the non-infarcted area ($p = 0.9$). Interestingly, there was a significant correlation between Gal-3 protein expression and macrophage infiltration (CD-68) in both infarcted ($r = 0.837$; $p < 0.001$) and non-infarcted areas ($r = 0.765$; $p < 0.001$). In the infarcted area, Gal-3 mRNA expression presented a positive and statistically significant correlation with mRNA expression of Col I ($r = 0.507$; $p < 0.001$), Col III ($r = 0.413$; $p = 0.007$) and TIMP-1 ($r = 0.837$; $p < 0.001$). In the non-infarcted area, Gal-3 mRNA expression correlated with TIMP-1 ($r = 0.542$; $p < 0.001$) but we did not find any significant correlation with Col I ($p = 0.698$) or Col III ($p = 0.852$). The same correlations were found for protein levels of Gal-3.

In conclusion, we found that Gal-3 increases in myocardial tissue in response to the occurrence of MI, and it is co-localized with macrophage infiltration. Interestingly, Gal-3 kinetic differs in infarcted versus remote areas: the greatest concentrations of Gal-3 were found early on in the infarcted area a peak 1 week after MI, which was correlated with the maximum expression of fibrosis markers. However, a later up-regulation of Gal-3 was demonstrated in remote areas not involved with MI. The up-regulation of Gal-3 in non-infarcted areas correlated with macrophage infiltration and TIMP-1, but not with the expression of collagens. These differences in Gal-3 timing depending on the location, infarcted and remote areas, might suggest different pathophysiologic participation of Gal-3 in the processes of cardiac remodeling after MI and the progression to overt HF. Recently, experimental data have suggested disruption of Gal-3 or pharmacological inhibition of Gal-3 as possible therapeutic strategies in cardiac remodeling and HF [7,8]. Our results provide knowledge about the temporal changes of Gal-3 expression in cardiac tissue in the weeks following a MI and suggest a different pathophysiologic role of Gal-3 in cardiac remodeling in infarcted and non-infarcted regions. These data may be useful for the development of future strategies aimed to inhibit Gal-3 expression and signaling as therapy for MI and HF progression. Further experimental approaches aimed to target Gal-3 are necessary to definitely understand the mechanistic or regulatory role of Gal-3 in the pathophysiology of MI and progression to HF.

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