Cardiovascular Pathology 25 (2016) 208–213

Contents lists available at ScienceDirect

Cardiovascular Pathology

Original Article

# Early administration of Enalapril prevents diastolic dysfunction and ventricular remodeling in rabbits with myocardial infarction

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ARTICLE INFO

*Article history:*
Received 6 November 2015
Received in revised form 15 January 2016
Accepted 16 January 2016
Available online xxxx

*Keywords:*
Myocardial infarction
Enalapril
Ventricular remodeling
Diastolic function

ABSTRACT

We aimed to investigate the role of early administration of Enalapril (Enal) on post-myocardial infarction (MI) ventricular remodeling and diastolic dysfunction in rabbits. White New Zealand rabbits that underwent coronary artery ligature or Sham were divided in three experimental groups: (1) Sham, (2) MI, and (3) MI + Enal. Enal was given by gavage at a dose of 10 mg/kg/day starting at 3 h after surgery for 35 days. At the end of the protocol, we measured (1) mean arterial pressure, (2) left ventricular (LV) + $dP/dt_{max}$, (3) LV end-diastolic pressure (LVEDP) and isovolumic relaxation (Tau), (4) LV dimensions, (5) LV ejection and shortening fraction, (6) infarct size (Masson's trichrome-stained slices), (7) fibrosis in the infarct and remote zone (Picrosirius red-stained slices), and (8) myocyte cross-sectional area (MCSA) in WGA-stained section. Enal reduced the mean arterial pressure by 30% as compared with untreated animals and Sham ($P<.05$). MI reduced LV + $dP/dt_{max}$ and LV − $dP/dt_{max}$ (mmHg/s), increased LVEDP (mmHg), Tau (ms), and $t_{50}$ (ms) values, suggesting a decrease in the relaxation rate. LV end-diastolic dimension and LV end-systolic dimension (LVESD, mm) increased in untreated MI ($P<.05$ vs. Sham). In contrast, Enal markedly prevented post-MI diastolic dysfunction by significantly decrease LVEDP from 8.2 ± 0.2 to 5.1 ± 0.3 mmHg, Tau from 19.8 ± 0.8 to 15.3 ± 0.9 ms, and $t_{50}$ from 12.4 ± 0.5 to 9.6 ± 0.8 ms as well as reduced LVESD from 15 ± 1.1 to 12 ± 0.8 mm ($P<.05$ MI vs. MI + Enal). Collagen concentration in the scar was unaffected, but chronic treatment with Enal prevented myocardial fibrosis and MCSA in the remote zone. In summary, chronic early administration of Enal to rabbits with experimental MI has a favorable effect on ventricular remodeling and diastolic function by reducing MCSA and fibrosis, without affecting the wound healing.



## 1 Introduction

After myocardial infarction (MI), a healing process initiates and the heart begins a remodeling process leading to dysfunction and failure. Therefore, an early therapeutic intervention after an infarction remains the cornerstone for the treatment of this pathology since it can revert its unfavorable evolution. It is also widely accepted that early activation of the renin-angiotensin system (RAS) through its main effector, angiotensin II (Ang II), contributes in the development of adverse remodeling and failure [1,2], also promoting the healing process as a physiological mechanism for replacement of necrotic cells by a scar. The use of Ang II blockers or angiotensin conversion enzyme inhibitors has been widely used to prevent adverse remodeling and the development of heart failure [3,4]. Although it is recommended to initiate treatment with angiotensin converting enzyme (ACE) inhibitors from the onset of MI, the role of these drugs to prevent adverse remodeling and diastolic dysfunction is still under discussion [5,6]. Furthermore, it should be considered that the cardiac expression of the RAS components varies depending on the species and can modify the response to these drugs. Clinical and experimental studies have demonstrated that ACE inhibitors improved ventricular remodeling and survival after an MI [7–9], but it is not clear if this beneficial effect includes an improvement of diastolic dysfunction. We have previously showed that the expression of the RAS components in rabbits with MI differs from those described for other species and that chronic treatment with Losartan adversely modified left ventricular (LV) remodeling in hearts with MI [10]. Accordingly, it would be of clinical interest to know whether the early administration

Grants: This work was supported by (1) National Agency of Scientific and Technological Promotion Grants PICT 2013-0373 (RJG) and PICT 2014-2320 (GEG), (2) National Council of Scientific and Technological Research Grant PIP11220130100012 (RJG), and (3) University of Buenos Aires Grant UBACyT 2013-2016 20020120200031 (GEG).

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http://dx.doi.org/10.1016/j.carpath.2016.01.004
1054-8807/

of ACE inhibitors, whose mechanism of action differs AT1 blockers, in the same experimental conditions modifies differently post-MI remodeling and function. Therefore, the goal was to study the effects of early administration of ACE inhibitors on remodeling and ventricular diastolic function in rabbits with MI.

## 2 Methods

### 2.1 Experimental model of MI

White New Zealand rabbits (body weight: 2.0–2.3 kg) were anesthetized with a ketamine (75 mg/kg) and xylazine (0.75 mg/kg), then intubated and mechanically ventilated using a Harvard ventilator (tidal volume: 25 ml) at a respiratory frequency of 34–38 cycles/min, as was previously described [10]. Subsequently, a lateral left thoracotomy followed by a pericardectomy and ligature of a lateral branch of the left coronary artery using a 6.0 silk thread were performed. Finally, the chest was closed in layers, and the animals were allowed to recover from the anesthesia in a quiet environment. Sham-operated animals underwent the same procedure without ligature of the coronary artery. After the animals recovered from the anesthesia, they were housed in individual cages until the end of the protocol. All experiments were approved by the Animal Care and Research Committee of the University of Buenos Aires, and this investigation conforms to the guidelines from the American Physiological Society "Guiding Principles in the Care and Use of Laboratory Animals."

### 2.2 Protocols and experimental groups

Three experimental groups were performed ($n = 5$–10). Animals were randomized according to the following groups: (1) Sham, (2) MI, and (3) MI + Enalapril (Enal). All the animals were followed up for 35 days. Enal was administered by gavage at a dose of 10 mg/kg/day.

### 2.3 Echocardiography

At the end of the protocol, rabbits were weighed and anesthetized with ketamine and xylazine as described above. LV dimensions (wall thickness, cavity dimensions, and areas either in systole or diastole) and ventricular function [ejection fraction (EF) (%), shortening fraction (SF) (%), and cardiac output (ml/min)] were evaluated with a Doppler echocardiography system equipped with an 8-MHz linear transducer (Acuson c256).

### 2.4 Arterial and cardiac catheterization

Arterial blood pressure and LV function were recorded by using a catheter placed inside of the femoral artery and another catheter placed in the carotid artery and advanced to left ventricle [11]. We measured systolic and diastolic ventricular pressures and its derivative in real time. This data was recorded on a PC provided with platelet analog–digital converter (National Instruments) and software for this purpose.

### 2.5 Histomorphometric analysis

#### 2.5.1 Quantitative determination of infarct size

After functional determinations, hearts were arrested in diastole with 2 M KCl. The balloon was then refilled with water until it reached a final physiological pressure (10–12 mmHg). Then, hearts were perfused with 10% formaldehyde (pH 7.2), allowing 5 min for fixation, and then remained in formaldehyde with the same volume for 72 h. Hearts were cut in slices from apex to base. Slices from a middle section of the hearts were paraffin embedded, and 5-mm-thick sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome. Slices stained with Masson's trichrome were scanned, and the infarct size was calculated from planimetric measurements using Image Pro-Plus 6.0 software (Media Cybernetics, Silver Spring, MD). The infarct size was calculated as the total length of the scar as a percentage of the total LV circumference, using the average of endocardial and epicardial tracings.

#### 2.5.2 Histology

Hearts from each group at 35 days after surgery were used for histological analysis. After death, hearts were excised from the thorax and immersed in 10% formaldehyde for 72 h. Later, hearts were cut from apex to base and embedded in paraffin, 5-mm serial cuts were made, and sections were stained with H&E and Picrosirius red. Myocyte cross-sectional areas were determined on digitalized images of rhodamine-conjugated lectin-stained sections (WGA no. RL-1022; Vector Laboratories, Burlingame, CA) of paraffin-embedded samples. These digitalized images were obtained using a fluorescence microscope (Olympus BX61) attached to a digital camera and connected to a computer equipped with image analysis software. Outlines of myocyte were traced, and cell areas were measured with Image Pro-Plus 6.0. At least 80 measurable cross-sections of myocyte from the septum were routinely measured [12,13]. In slices stained with Picrosirius red, interstitial collagen deposition was also measured in the septum and scar using the image analysis system described above. The percentage of collagen for each region was calculated by adding the areas corresponding to collagen and dividing by the addition of the areas corresponding to myocyte plus the areas of collagen tissue.

### 2.6 Statistical analysis

All values are expressed as mean ± S.E.M. One-way ANOVA followed by the Newman–Keuls posttest was used for comparing individual differences in arterial blood pressure, cardiac function and also for morphometric and histological measurements. $P < .05$ was considered statistically significant.

## 3 Results

Table 1 shows the general data and the MI size at 5 weeks of evolution. Permanent ligature of the coronary artery produced an infarct that affected 30% of the LV mass in MI and 34% in animals with MI chronically treated with Enal ($P$ = NS). LV mass was between 3.2 ± 0.2 and 2.7 ± 0.2 and no significant difference among the groups was observed.

Mean arterial blood pressure (MBP) remained unchanged in the group of animals with MI compared to Sham, while treatment with Enal reduced MBP by 30% in the group of animals with MI (Fig. 1A, $P < .0.05$ vs. MI). Fig. 1B–F shows ventricular function assessed by cardiac catheterization. MI significantly reduced contractility evaluated by $+dP/dt_{max}$ (Fig. 1B). Furthermore, diastolic function assessed by LV end-diastolic pressure (LVEDP), $-dP/dt_{max}$, $t_{50}$, and Tau was clearly impaired in MI group ($P < .05$ MI vs. Sham). However, Enal administration improved diastolic function by decreasing LVEDP (from 8.2 ± 0.2 to 5.1 ± 0.3 mmHg) and increasing the isovolumic relaxation rate as evaluated by $t_{50}$ (from 12.4 ± 0.5 to 9.6 ± 0.8 ms) and Tau (from 19.8 ± 0.8 to 15.3 ± 0.9 ms) indices (Fig. 1C–F, $P < .05$ MI vs. MI + Enal).

We have observed by echocardiography that EF and SF significantly decreased in animals with MI, as it was expected, and this drop was

**Table 1**
Body weight (BW), LV weight, and infarct size (%)

| | Body weight (kg) | LV weight (g) | LV weight/BW (g/g) | Infarct size (%) |
|---|---|---|---|---|
| **Sham** | 2.8 ± 0.1 | 8.7 ± 0.4 | 3.2 ± 0.2 | – |
| **MI** | 2.8 ± 0.1 | 8.3 ± 0.4 | 2.8 ± 0.3 | 30 ± 3.0 |
| **MI + Enal** | 2.6 ± 0.1 | 6.9 ± 0.4* | 2.7 ± 0.2 | 34 ± 8.1 |