

Basic Science and Experimental Studies

Eplerenone, a Selective Aldosterone Blocker, Improves Diastolic Function in Aged Rats With Small-to-Moderate Myocardial Infarction

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ABSTRACT

Background: The incidence of cardiovascular diseases increases rapidly with age, and the elderly suffer higher morbidity and mortality. Aldosterone blockers have shown benefits in patients with left ventricular (LV) dysfunction and heart failure after myocardial infarction (MI). However, aldosterone blockade efficacy has not been explored in aged animals with MI.

Methods and Results: Small-to-moderate MI was induced by coronary artery ligation in 16-month old rats, divided into 3 groups: sham-operated (control, n = 9), MI (n = 9), and MI fed a diet containing eplerenone (120 mg/kg/day, MI+Eplerenone, n = 9) given 18 days postsurgery and up to sacrifice 3 months later. At sacrifice, untreated MI rats did not show overt systolic dysfunction but they had (1) echocardiographic evidences of impaired relaxation (increase of E wave deceleration time and of isovolumic relaxation time, decrease of peak E wave velocity), (2) hemodynamically impaired LV relaxation (LV $-dP/dt$ from 7413 ± 720 to 4956 ± 475 mm Hg/s, $P < .05$), and (3) significant increase of collagen content in LV interstitium (from 4.27 ± 0.23 to $5.34 \pm 0.24\%$, $P < .01$) and in aorta (from 19 ± 1 to $24 \pm 2\%$, $P < .05$). Eplerenone normalized echocardiographic and hemodynamic evidences of diastolic dysfunction, as well as myocardial interstitial collagen and aortic fibrosis (all parameters statistically different from untreated MI).

Conclusion: In aged rats with small to moderate MI, eplerenone normalized diastolic relaxation, possibly through a reduction of interstitial fibrosis.

Key Words: Aging, myocardial infarction, aldosterone, echocardiography, diastolic function, aorta.

The incidence of cardiovascular diseases increases with age, and the elderly suffer higher morbidity and mortality from cardiovascular diseases.¹ Myocardial changes with

aging have been best studied in small rodents in which progressive alterations in the structure and function are used as a model of aging in "healthy" humans, and a complex pattern of structural, functional, and molecular changes has been depicted.² Briefly, progressive loss of cardiac myocytes, compensated by hypertrophy and increased deposition of connective tissue occur with aging. These cellular changes, together with multilevel molecular alterations in calcium handling, contribute to a progressive impairment of cardiac relaxation.²

Longitudinal studies in carefully selected populations of healthy subjects have shown age-associated changes in their diastolic filling pattern.^{3,4} These observations recall echocardiographic findings in small rodents that document an age-associated decline in diastolic function, whereas systolic function is relatively preserved in nonsenescent rats.^{5,6}

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Few data are available on the effects of an acute cardiac injury such as myocardial infarction (MI) when it occurs on a substrate already altered by aging. Chronic activation and imbalance among the neurohormonal systems that regulate cardiac physiology contribute to the progressive deterioration of cardiac function after MI and heart failure (HF). Drugs that reduce this excessive neurohormonal tone are beneficial in these patients. In particular, aldosterone receptor blockers reduce mortality and morbidity in large-scale clinical trials in patients with severe HF⁷ or left ventricular (LV) dysfunction and HF after MI.⁸ Though the molecular targets and mechanisms are far from being fully elucidated, aldosterone exerts numerous detrimental effects on the heart and plays a critical role in myocardial remodeling.⁹

This study was undertaken to evaluate the efficacy of eplerenone, a selective aldosterone blocker in aged rats with small-to-moderate MI induced by permanent coronary artery occlusion. The number of reports published using this model is limited¹⁰⁻¹³ and, to the best of our knowledge, do not include drug evaluations, despite clear clinical relevance to the elderly population.

Materials and Methods

Experimental Design

Forty male Wistar rats (Harlan, S. Pietro al Natisone, Italy), age 16 months (body weight 626 ± 10 g), were subjected to coronary artery ligation or sham-operation. Ten days after surgery, echocardiographic examinations were performed to identify rats with evidences of regional wall motion abnormalities that were divided into 2 experimental groups (MI), and MI+Eplerenone. A group of sham-operated animals served as control ($n = 9$ animals for each group). Treatment was initiated 18 days after surgery and lasted until sacrifice (3 months). Immediately before sacrifice, echocardiographic and hemodynamic examinations were performed.

Surgery

Small-to-moderate MI was induced by ligation of left descending coronary artery in animals under equithesin anesthesia (3 mL/kg of body weight) and electrocardiogram monitoring, as previously reported.¹⁴ The position of the ligature along the left anterior coronary artery tree (approximately halfway between LV base and apex) was chosen to yield MI of small to moderate size because all rats with large MI ($n = 5$) died in a pilot experiment. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., Suppl 40, 18 Febbraio 1992, Circolare n. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec 12, 1987; Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996).

Drug Administration

Eplerenone was supplemented in the chow of the MI+Eplerenone group, whereas a control diet without drug was given to the control and MI groups (Research Diets Inc, New Brunswick, NJ). The average intake of eplerenone was 120 mg/kg body weight per day, based on drug concentration in the pellets (approximately 3000

ppm) and chow intake. Water and chow were given ad libitum. Body weight and chow intake were monitored weekly throughout the study.

Echocardiography

Echocardiograms were recorded under light sedation (diazepam/xylazine 10/1.25 mg/kg intraperitoneally) with a commercial system (Aloka SSD-5500, Aloka, Tokyo, Japan) equipped with 13 MHz linear and 7.5 MHz phase array ultrasound transducers. Two-dimensional (2D) parasternal long-axis images were acquired so the maximum LV length could be identified. Short-axis images were obtained at the papillary muscle level and 2D guided M-mode tracing were recorded at a speed of 100 mm/s. Anterior and posterior end-diastolic and end-systolic wall thickness and LV internal dimensions were measured according to the American Society for Echocardiography leading-edge method. LV end-diastolic (LVEDV), end-systolic volumes (LVESV), and ejection fraction (LVEF) were calculated by using the modified Simpson's single plane rule from long-axis view. End-diastole was selected as the largest LV area and end-systole as the smallest.¹⁵

Regional wall motion (WM) abnormality was assessed through visual determination of akinetic or dyskinetic (A/D) segments evaluating systolic thickening with repeated real-time video playback. A $WM_{A/D}$ index was calculated expressing the number of A/D segments as a percentage of the number of segments visualised.¹⁶

Pulsed-wave Doppler spectra of mitral inflow were obtained from apical 4-chamber view. The sample volume was placed at the tip of the mitral leaflets and adjusted to the position of maximal velocity. The peak of early (E) and late filling waves (A), E wave deceleration time (DT), and isovolumic relaxation time (IVRT) were measured. LV outflow velocity was recorded from the apical 5-chamber view obtained by further angulation of the transducer. Aortic systolic velocity time integral (VTI) and aortic root diameter were determined and stroke volume (SV) and cardiac output (CO) calculated according to the formula:

$$SV = D^2 \times VTI \times 0.785, \text{ where } D \text{ is the aortic diameter;}$$

$$CO = SV \times \text{heart rate}$$

All Doppler spectra were recorded for 4 to 8 cardiac cycles at a sweep speed of 100 mm/s. Images were digitally acquired and stored for offline analysis by an experienced sonographer blinded to the treatment groups.

Hemodynamics

Hemodynamics was performed under light preanesthesia (ketamine/xylazine 25/5 mg/kg) followed by anesthesia with isoflurane 1.5% in N₂O:O₂ 2:1. A microtip pressure transducer (Millar SPC-320, Millar, Houston, TX) connected to a recorder (Windograf, Gould Electronics, Valley View, OH) was inserted into the right carotid artery to record systolic (SBP) and diastolic blood pressure (DBP). The pressure transducer was then advanced into the LV to measure LV systolic (LVSP) and end-diastolic (LVEDP) pressures, the first derivative of LV pressure over time (+dP/dt and -dP/dt), and heart rate.

Biohumoral Analyses

The animals were individually housed in metabolic cages for 24 hours to collect urine after 11 weeks and measure the excretion

of Na⁺ and K⁺ with selective electrodes, aldosterone (RIA DiaSorin) and creatinine (colorimetric Jaffé assay). Blood was collected before sacrifice to measure plasma aldosterone (RIA DiaSorin, Saluggia, Italy) and renin activity (RIA Perkin Elmer, Monza, Italy).

Histology

Immediately after hemodynamic measurements, the heart was arrested in diastole, excised, and weighed. A transversal section of the LV taken at the level of the infarct was fixed in phosphate-buffered 4% formaldehyde, embedded in paraffin, and 5- μ m sections stained with hematoxylin-eosin to measure infarct area, myocyte length, and cross-sectional area. Infarct size was expressed as percentage of infarct area over the LV area by tracing the perimeter manually. The areas were computed by an image analyzer (AIS, Analytical Imaging Station, version 3.0, Imaging Research Inc., St. Catharine's Canada). Myocyte length was calculated by morphometric analysis.¹⁷ Interstitial and perivascular collagen densities were measured on Sirius red-stained sections.^{14,18} A segment of the descending thoracic aorta was excised, fixed in phosphate-buffered 4% formaldehyde, embedded in paraffin, and 10- μ m sections stained with Sirius red for collagen fiber staining or orcein for elastin.¹⁹ Collagen or elastin content in the aortic media were measured with a semiautomated image analysis software (Kontron KS300, Kontron, Venegono, Italy). The medial density of collagen or elastin were averaged in 16 fields per section under a magnification of 400 \times and expressed as relative density. Kidneys were excised, weighed, and cut longitudinally; half was fixed in phosphate-buffered 4% formaldehyde, embedded in paraffin, and 5- μ m sections stained with hematoxylin-eosin for qualitative histologic evaluation by an experienced histologist blinded to treatment groups.

Myocardial Gelatinase Activity

Gelatinase activity was measured with a commercial kit (Chemicon, Temecula, CA) with purified MMP-2 and MMP-9 as standards and expressed as total activity (ng/mL) in the infarcted tissue and spared interventricular septum.

Statistical Analysis

Data are presented as mean \pm SEM. Differences between groups were evaluated by 1-way analysis of variance (ANOVA) followed by Dunnett's posttest with the MI group as reference. Values of $P < .05$ were considered significant.

Results

Gross Characteristics of the Aged Rats

Mortality averaged 40% (12/30) in the rats with ligation of coronary artery. One rat of the control (sham) group died before sacrifice. At sacrifice, there were no significant differences in body and cardiac weights (LV and RV free wall) among these groups (Table 1). Infarct area was similar in the MI (9.0 \pm 2.8% LV area) and MI+Eplerenone groups (10.8 \pm 1.8% LV area, $P = .59$). Kidney weight was significantly smaller in the rats receiving eplerenone.

Table 1. Gross Characteristics at Sacrifice

	Control	MI	MI+Eplerenone	1-way ANOVA (P)
Body weight (g)	596 \pm 13	595 \pm 14	568 \pm 13	.27
Heart weight (mg)	1376 \pm 24	1326 \pm 48	1419 \pm 34	.22
LV weigh (mg)	946 \pm 17	924 \pm 31	972 \pm 27	.43
RV free wall weight (mg)	213 \pm 7	216 \pm 12	220 \pm 7	.87

MI, myocardial infarction; ANOVA, analysis of variance; LV, left ventricular; RV, right ventricular.

Echocardiographic Analyses

Regional wall motion abnormalities. A representative echocardiographic examination of the animals before sacrifice, at the age of 19 months (3 months after surgery), is shown in Fig. 1. Wall motion abnormalities were most frequently observed at mid anterolateral and anteroapical segments in both MI and MI+Eplerenone groups. WM_{A/D} index was similar in the 2 groups with MI at baseline and remained unchanged until sacrifice (ie, 20.5 \pm 2.0 and 21.5 \pm 1.6 % of the LV in the MI and MI+Eplerenone groups, respectively) (Table 2).

Systolic function and LV dimensions. LV ejection fraction was slightly reduced in both the MI (58 \pm 4%) and MI+Eplerenone groups (60 \pm 5%) compared with control animals (72 \pm 3%, $P < .05$, Table 2). At similar heart rate in the 3 study groups, there was a tendency for LV chamber enlargement in the rats with MI and MI+Eplerenone.

Diastolic function. The aged control rats had low peak E velocity, high peak A velocity and consequently low E/A ratio compared with younger animals. The peak E wave velocity decreased (from 73 \pm 3 to 63 \pm 5 cm/s, $P > .05$, Fig. 2a), whereas E wave deceleration time (DT) increased (from 42 \pm 3 to 61 \pm 5 ms, $P < .01$, Fig. 2b) from control to untreated rats with MI. Similarly the IVRT tended to increase, from 22 \pm 2 to 28 \pm 3 ms (Fig. 2c). Eplerenone significantly restored these parameters to levels comparable with those observed in control animals (E_{vel} = 78 \pm 5 cm/s, DT = 46 \pm 5 ms, IVRT = 20 ms, Fig. 2).

Invasive Hemodynamics

At sacrifice, systemic arterial blood pressure was significantly lower in the MI group (SBP/DBP = 102/73 mm Hg) than in the control group (124/88 mm Hg, $P < .05$ for both, Table 3). Eplerenone did not significantly modify these pressures. LV systolic and end-diastolic pressures were not significantly altered in aged rats with MI. The negative derivative of LV pressure over time (LV $-dP/dt$) was depressed by 33% in rats with MI (4956 \pm 475 mm Hg/s) compared with controls (7413 \pm 720 mm Hg/s). Eplerenone tended to normalize this parameter (6811 \pm 730 mm Hg/s, $P > .05$).

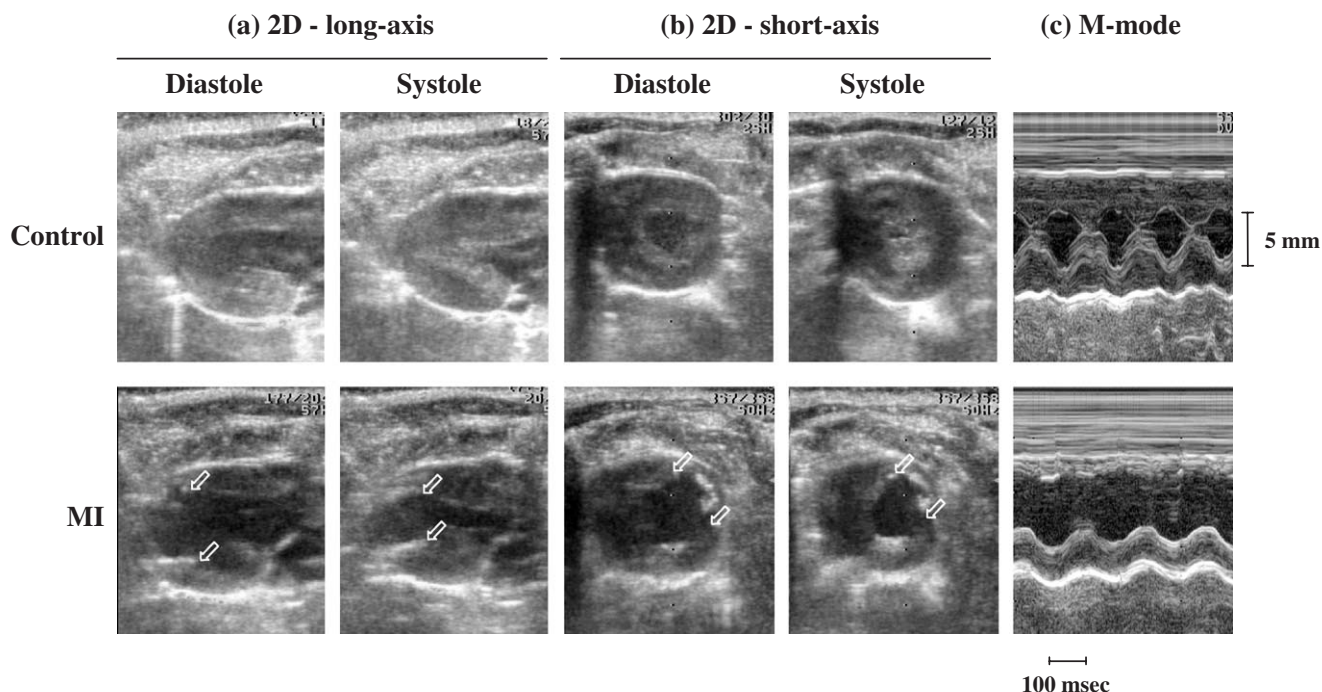


Fig. 1. Representative echocardiographic examinations. Two dimensional long-axis (a) and short-axis views (b), and M-mode tracings (c) from a control rat (upper panels) and a rat with myocardial infarction (lower panels). Arrows mark junctions between akinetic segments and contracting myocardium. M-mode tracing from short axis view shows the degree of anterior wall thinning and akinesis after infarction.

Histologic Evaluation of Myocardium, Aorta, and Kidneys

The average myocyte cross-sectional area was 393 ± 9 , 372 ± 7 , and $375 \pm 9 \mu\text{m}^2$ in the control, MI, and MI+Eplerenone groups (ANOVA $P = .20$), whereas corresponding values for myocyte length were 117 ± 4 , 111 ± 6 , and $117 \pm 15 \mu\text{m}$ (ANOVA $P = .89$). There was a moderate (25%) but significant increase in interstitial collagen deposition in the spared septal myocardium in the rats with MI ($5.34 \pm 0.24\%$

area) compared with controls ($4.27 \pm 0.23\%$ area, $P < .01$ versus MI, Fig. 3). Eplerenone normalized interstitial fibrosis ($4.45 \pm 0.13\%$ area, $P < .05$ versus MI). In contrast, perivascular fibrosis in the septum was similar among the 3 experimental groups ($0.78 \pm 0.06\%$, $0.72 \pm 0.08\%$, and $0.84 \pm 0.08\%$ of coronary vessel area, in the control, MI and MI+Eplerenone groups, respectively; ANOVA $P = .55$).

Collagen density in the media of thoracic aorta in the MI group was increased by 26% (from $19 \pm 1\%$ to $24 \pm 2\%$,

Table 2. Echocardiographic Data at Baseline (10 days After CAO) and Sacrifice (3 months After CAO)

	Control	MI	MI+Eplerenone	1-way ANOVA (<i>P</i>)
		<i>Baseline</i>		
Heart rate (bpm)	342 ± 21	323 ± 28	348 ± 22	.73
WM _{A/D} index (%)	0	18.5 ± 2.0	21.5 ± 1.6	—
LV ejection fraction (%)	72 ± 3	$54 \pm 5^{**}$	53 ± 3	.0023
		<i>3 months</i>		
Heart rate (bpm)	322 ± 21	311 ± 29	328 ± 20	.87
WM _{A/D} index (%)	0	20.5 ± 2.0	21.5 ± 1.6	—
LV shortening fraction (%)	56 ± 2	$48 \pm 2^*$	41 ± 3	.0012
LV ejection fraction (%)	73 ± 2	$58 \pm 4^*$	60 ± 5	.0023
Stroke volume (mL)	0.31 ± 0.02	0.28 ± 0.02	0.30 ± 0.04	.70
LV diastolic internal diameter (mm)	7.0 ± 0.2	7.3 ± 0.3	7.8 ± 0.2	.10
LV systolic internal diameter (mm)	3.1 ± 0.2	3.8 ± 0.3	4.6 ± 0.4	.0053
LV end-diastolic area (mm ²)	0.78 ± 0.03	0.80 ± 0.05	0.85 ± 0.04	.50
LV end-systolic area (mm ²)	0.36 ± 0.03	0.48 ± 0.04	0.53 ± 0.05	.018
Interventricular septum thickness (mm)	1.91 ± 0.01	1.88 ± 0.03	1.88 ± 0.03	.51
LV end-diastolic volume (mL)	0.36 ± 0.03	0.37 ± 0.03	0.42 ± 0.03	.38
Cardiac output (mL/min)	111 ± 12	86 ± 9	108 ± 17	.34

CAO, WM_{A/D} index, index of wall motion abnormality. All other abbreviations, see Table 1.

* $P < .05$.

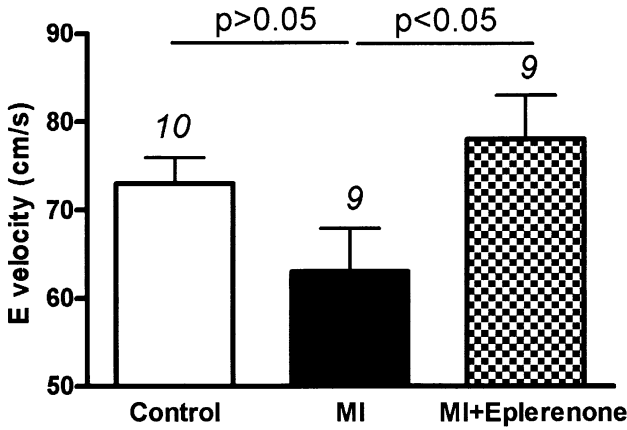
** $P < .001$ vs. control (Dunnett's posttest).

Table 3. LV and Systemic Hemodynamics at Sacrifice

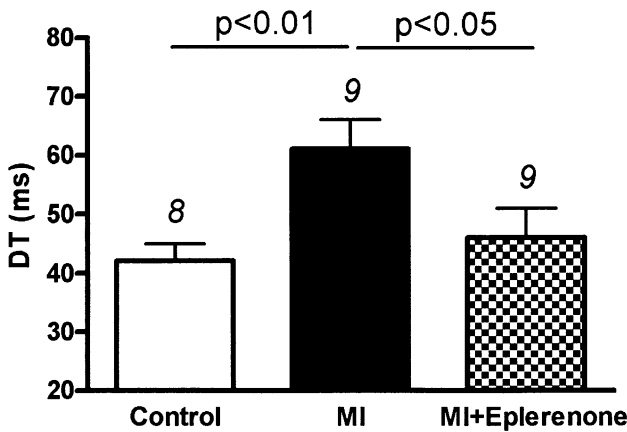
	Control	MI	MI+Eplerenone	1-way ANOVA (P)
SBP (mm Hg)	124 ± 7	102 ± 6*	108 ± 4	.0029
DBP (mm Hg)	88 ± 5	73 ± 5*	79 ± 3	.050
HR (bpm)	295 ± 12	256 ± 14	275 ± 13	.15
LVEDP (mm Hg)	10 ± 1	12 ± 1	10 ± 2	.64
LVSP (mm Hg)	119 ± 8	101 ± 6	110 ± 5	.17
LV +dP/dt (mm Hg/s)	8213 ± 892	6879 ± 710	7700 ± 605	.45
LV -dP/dt (mm Hg/s)	7413 ± 720	4956 ± 475*	6811 ± 730	.035

LV, left ventricular; MI, myocardial infarction; ANOVA, analysis of variance; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVEDP, LV end-diastolic pressure; LVSP, LV systolic pressure. **P* < .05 vs. control (Dunnett's posttest).

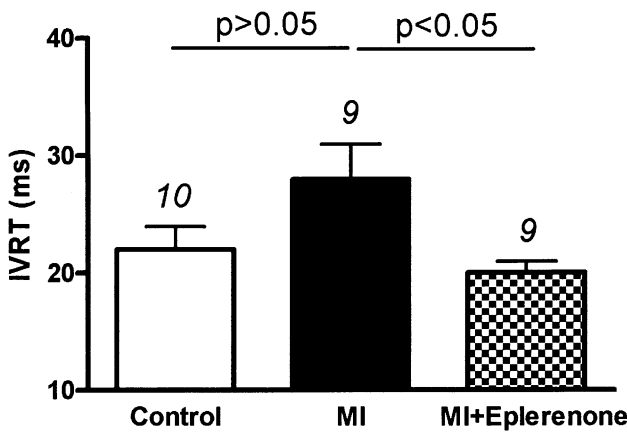
a) E wave velocity



b) E wave deceleration time



c) Isovolumic relaxation time



ANOVA *P* = .0033, control versus MI: *P* < .05) and elastin decreased by 23% (from 35 ± 1% to 27 ± 1%, ANOVA *P* < .0001, control versus MI: *P* < .05) compared with controls (Fig. 4). The density of collagen (16 ± 2%) and elastin (32 ± 1%) was almost normalized in the media of aorta in the MI+Eplerenone group. Consequently, the elastin to collagen ratio, depressed by 40% in the MI group, was similar to control level in the MI+Eplerenone group (ANOVA *P* = .0027, Fig. 4c). There were no differences in media wall thickness among the 3 experimental groups: 147 ± 8 μm (control), 149 ± 4 μm (MI), and 152 ± 6 μm (MI+Eplerenone), respectively (ANOVA *P* = .83).

Most kidneys presented multifocal chronic lesions characterized by intratubular proteinaceous casts, atrophy or regeneration of tubules, fibrosis, and interstitial lymphocytes infiltration. These lesions were consistent with chronic progressive nephrosis, typical of aged rats. However, the incidence and magnitude of degenerative lesions were not different among the 3 experimental groups.

Gelatinase Activity

There were no significant differences among the 3 groups for total gelatinase activity in the infarcted area (ANOVA *P* = .55) or in the septum (ANOVA *P* = .40).

Biohumoral Profile

There was no difference between control and MI groups for urinary creatinine, aldosterone, and electrolytes (Na⁺ and K⁺) excretion, and for plasma aldosterone concentration (Table 4). Eplerenone increased both aldosterone urinary

Fig. 2. Echocardiography-derived diastolic parameters from control rats, rats with myocardial infarction (MI), and MI rats treated with eplerenone (MI+Eplerenone). Data are mean ± SE for the number of animals indicated on top of bars. Analysis of variance: E wave velocity, *P* = .033; E wave deceleration time, *P* = .0099; isovolumic relaxation time, *P* = .050.

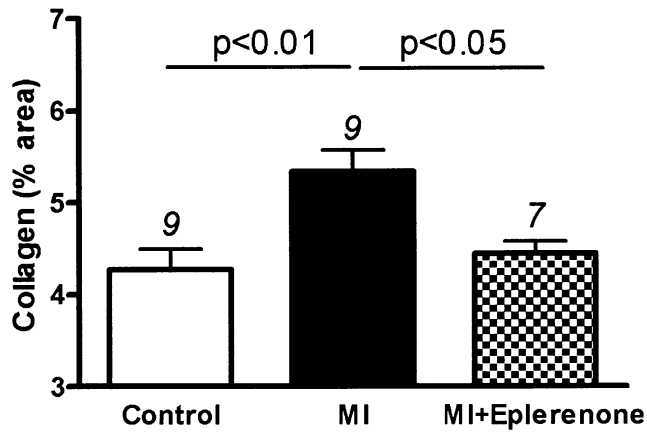


Fig. 3. Interstitial collagen deposition in the spared myocardium from control rats, rats with myocardial infarction (MI), and MI rats treated with eplerenone (MI+Eplerenone). Collagen fibers were stained with Sirius red. Interstitial fibrosis was quantified in the interventricular septum with automated image analysis and expressed as percent of tissue area. Data are mean \pm SE for the number of animals indicated on top of bars. Analysis of variance $P = .0034$.

excretion (2.6-fold, $P < .01$) and its plasma concentration (5-fold, $P < .01$). Plasma renin activity was also increased in MI+Eplerenone rats compared to MI rats (+45%, $P < .01$).

Discussion

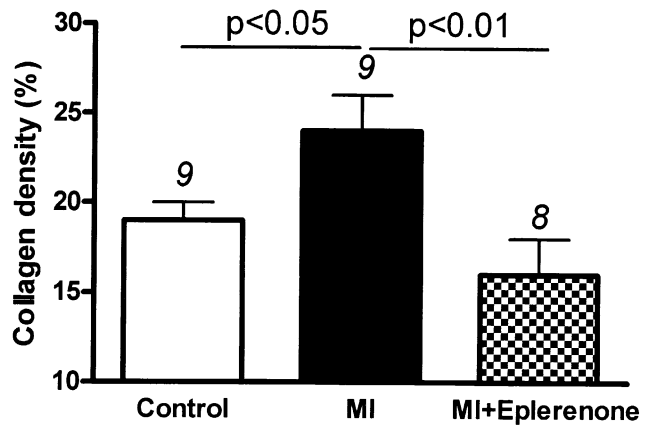
In aged rats with small-to-moderate MI, we report the following.

1. Small-to-moderate infarctions from coronary artery ligation in aged rats (19 months at sacrifice) induced mild systolic dysfunction and impaired diastolic relaxation after 3 months.
2. A specific aldosterone blocker, eplerenone, started 18 days after MI, improved LV relaxation as observed by independent echocardiographic and hemodynamic evaluation, accompanied by a reduction of interstitial fibrosis.
3. Eplerenone also normalized the elastin-to-collagen ratio in the media of thoracic aorta.
4. Long-term eplerenone therapy elicited the neurohormonal effects expected from its pharmacologic profile.

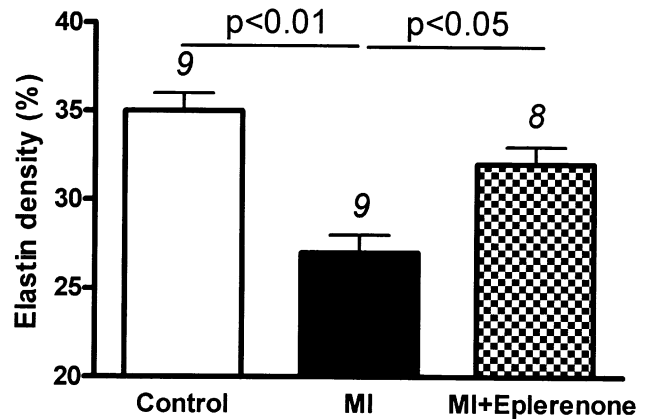
These effects of eplerenone after MI, already reported in young adult rats,^{20,21} are herein demonstrated for the first

Fig. 4. Collagen and elastin content in the thoracic aorta. The medial content of collagen and elastin was quantified with automated image analysis and expressed as percent of tissue area. Data are mean \pm SE for the number of animals indicated on top of bars. Analysis of variance: Collagen, $P = .033$; elastin, $P < .0001$; elastin/collagen, $P = .0027$.

a) Collagen



b) Elastin



c) Elastin/collagen

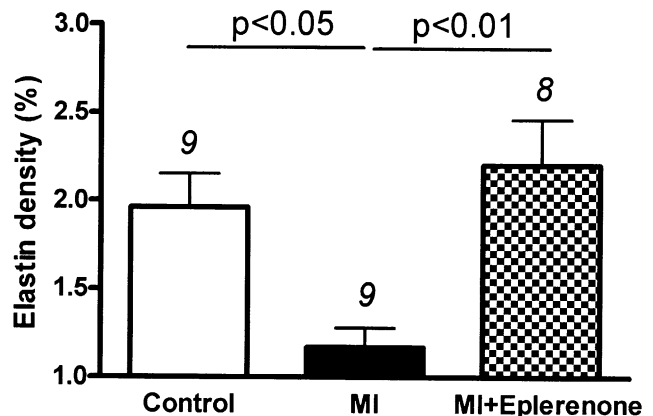


Table 4. Biohumoral Profile of Aged Rats With MI

	Control	MI	MI+Eplerenone	1-way ANOVA (<i>P</i>)
	<i>Urine</i>			
Log ₁₀ (Na ⁺ /K ⁺)	-0.31 ± 0.04	-0.25 ± 0.03	-0.27 ± 0.03	.53
Creatinine (mmol/kg/day)	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	.79
Aldosterone (ng/kg/day)	63 ± 5	65 ± 4	173 ± 19*	<.0001
	<i>Plasma</i>			
Aldosterone (pg/mL)	176 ± 48	167 ± 22	840 ± 93*	<.0001
PRA (ng/mL/h)	3.12 ± 0.47	3.46 ± 0.43	5.03 ± 0.49*	.017

PRA, plasma renin activity. See Table 1 for other abbreviations.

**P* < .01 vs. MI (Dunnett's posttest).

time in aged rats. In addition, these effects were observed with treatment started almost 3 weeks after MI, when the infarct was stable.

Aldosterone Activation in Cardiac Diseases

Besides its classical effects on electrolyte homeostasis, aldosterone exerts a series of deleterious effects on vital organs. The description of a steroidogenic system in the heart,²² and that cardiac aldosterone production is activated after MI in the rat²³ and in man,²⁴ have renewed experimental and clinical interests in pharmacologic blockade of aldosterone in cardiovascular diseases. Accordingly, aldosterone antagonists have shown significant clinical benefits in recently published clinical trials enrolling patients with HF⁷ or with LV dysfunction and HF after MI.⁸ Interestingly, ventricular, but not plasma renin-angiotensin-aldosterone system, is activated after MI in the rat.²³

LV Function in Aged Rats With MI

LV morphology and systolic function are relatively well preserved with aging, but cardiac relaxation declines progressively in the elderly, as the first expression of LV diastolic impairment.² In aged rats, Doppler echocardiographic studies show a comparable pattern, characterized by impaired relaxation and LV filling in early diastole with a normal systolic function.^{5,6} Hemodynamic data also show preserved systolic function (LV systolic pressure and +dP/dt) but altered diastolic relaxation (increase of time constant of isovolumic relaxation and end-diastolic pressure, decrease of -dP/dt).²⁵

To the best of our knowledge, the present report is unique because we investigated for the first time cardiac function by echocardiography in aged rats with MI and the impact of a drug in this model. Aged control rats showed a normal systolic function. The extent of myocardial damage induced by coronary artery ligation was intentionally limited here (approximately 10% of LV area) leading to a model of small-to-moderate apical MI with mild systolic dysfunction (EF decreased by 15%) and little evidence of overt LV remodeling after 3 months of untreated MI. However, impaired LV relaxation was clearly observed, as revealed by longer IVRT (+27%), reduced E wave peak velocity (*E_{vel}*) (-14%), and longer DT (+47%) compared with controls. These findings

were confirmed by independent hemodynamic analysis showing a significant reduction in LV -dP/dt by 33%.

Postinfarction Cardiovascular Responsiveness in Aged Rats

Small infarcts are associated with limited alterations of hemodynamic parameters, ventricular architecture, cellular and vascular adaptations.^{26,27} In the present study, MI elicited a moderate (25%) but significant increase in interstitial collagen deposition in the aged rats, of smaller extent to what expected in younger animals (in our hands, a 91% increase after MI has been found).²⁸ This may be due to the reduced capacity of aged fibroblasts to synthesize collagen *in vitro*²⁹ and *in vivo*³⁰ and is consistent with reduced reactive fibrosis in the aged rats after β -adrenergic stimulation¹⁸ or MI.¹³

We did not observe any hypertrophic response of the myocytes after MI. Myocytes were already enlarged in control aged rats (cross-sectional area 393 ± 9 μm^2 compared with 277 ± 9 μm^2 in younger animals)¹⁸; no further growth was observed in the 2 MI groups. Similarly, myocyte length was not increased in MI rats. These histologic observations are consistent with (1) echocardiographic data showing no increase in the thickness of septum and inferoposterior wall and (2) unchanged LV weight after these small-to-moderate MI. Attenuation of the hypertrophic response of cardiac myocytes after MI has been previously documented in aged rats after MI^{10,13} or isoproterenol stimulation,¹⁸ which confirms the lower cardiac responsiveness of these animals.

In the aged normotensive rat the thoracic aortic media wall becomes progressively thicker and collagen and elastin contents increase, resulting in a stiffer vessel.¹⁹ On the other hand, post-MI changes in the structure of large conduit arteries are rather limited in young animals, irrespective of infarct size.³¹ Here, we showed a significant increase of medial collagen content (+26%) and decrease of elastin (-23%) 3 months after small to moderate MI in aged rats. These changes are somewhat larger to those expected in younger animals. Eplerenone treatment normalized the elastin to collagen ratio in aortic media, suggesting a role for mineralocorticoid receptors in structural alterations of large vessels in aged rats after MI, as observed in aldosterone-induced hypertension³² or during normal aging.³³

Aldosterone Antagonism in Aged MI Rats

The benefits of aldosterone blockers on ventricular remodeling are well documented in young adult rats with MI.^{23,34} Here, eplerenone given to aged rats after MI did not improve LV contractility (mildly depressed), as already reported in adult rats^{20,21} or in dogs with HF from coronary microembolization.³⁵ In contrast, eplerenone was very effective in restoring LV relaxation parameters in the aged rats, as demonstrated independently by 3 echocardiographic (peak E velocity, DT, and IVRT) and 1 hemodynamic (LV $-dP/dt$) variables. These results are consistent with those of the Eplerenone Post-AMI Heart Failure Efficacy and Survival Study (EPHESUS) trial⁸ and suggest that eplerenone may provide benefit in elderly populations with diastolic dysfunction.

The exact mechanism for the benefit of eplerenone in these aged rats is still under investigation, but normalization of myocardial interstitial fibrosis may contribute to improved LV relaxation.³⁶ In younger animals, similar reductions in reactive collagen after MI by aldosterone antagonists have been observed with different molecules.^{21,23,34} However, we could not find any significant difference in myocardial gelatinase activity (MMP-2 and MMP-9) in our aged rats, as previously shown in dogs with HF.³⁵ It should be noted that myocardial expression of several MMPs is depressed during aging,³⁷ but nothing is known about their regulation after MI in aged animals. Further mechanisms, such as the anti-inflammatory properties of eplerenone,³⁸ are being explored.

Eplerenone monotherapy was assessed here for the first time in aged animals with MI. However, further evaluations of this drug in combination with recommended pharmacologic therapy for MI and HF (angiotensin-converting enzyme inhibitors, β -blockers) are required to reflect clinical practice more closely. The effects of eplerenone, which appears to be targeted to diastolic dysfunction in the aged rat with MI, might be a promising start for defining new therapies for HF with preserved systolic function.

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