Beneficial effects of delayed ivabradine treatment on cardiac anatomical and electrical remodeling in rat severe chronic heart failure

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Milliez P, Messaoudi S, Nehme J, Rodriguez C, Samuel JL, Delcayre C. Beneficial effects of delayed ivabradine treatment on cardiac anatomical and electrical remodeling in rat severe chronic heart failure. Am J Physiol Heart Circ Physiol 296: H435–H441, 2009. First published December 12, 2008; doi:10.1152/ajpheart.00591.2008.—We tested the hypothesis that heart rate (HR) reduction, induced by the selective hyperpolarization-activated current inhibitor ivabradine (Iva), might improve left ventricular (LV) function, structure, and electrical remodeling in severe post-myocardial infarction (MI) chronic heart failure (HF). MI was produced in adult male Wistar rats. After 2 mo, echocardiography was performed before the randomization into MI and MI + Iva (10 mg·kg−1·day−1) groups. After 3 mo of treatment, echocardiography and 24-h telemetry were recorded. Cardiac collagen, mRNA, and protein expressions of angiotensin-converting enzyme (ACE) and ANG II type 1 (AT1) receptor were quantified. As a result, at 2 mo post-MI, all rats displayed severe congestive HF signs (ejection fraction < 30%). At 5 mo post-MI, body and heart weights were similar in the MI and MI + Iva groups. LV ejection fraction and LV end-diastolic pressure were worsened in the MI group, whereas both were improved with Iva. Iva reduced HR by 10.4% (P < 0.03 vs. MI) and ventricular premature complexes by 89% (P < 0.03) and improved HR variability (standard deviation of the RR interval) by 22% (P < 0.05). There were no effects of Iva on PR, QRS, and QT durations. Interstitial fibrosis in the MI-remote LV was markedly reduced by Iva (4.0 ± 0.1 vs. 1.8 ± 0.1%, P < 0.005). Increases in ventricular gene and protein expressions of ACE and AT1 receptor in MI were completely blunted by Iva. In conclusion, these data indicated that HR reduction by Iva prevents the worsening of LV dysfunction and remodeling that may be related to a downregulation of cardiac renin-angiotensin-aldosterone system transcripts. Such beneficial effects of Iva on cardiac remodeling open new clinical perspectives for the treatment of severe HF.

heart rate; cardiac fibrosis; renin-angiotensin-aldosterone system

The results from several large clinical studies indicate that the optimal treatment of severe (New York Heart Association class III and IV) heart failure (HF) associates an angiotensin-converting enzyme (ACE) inhibitor, a β-blocker and an anti-aldosterone (± diuretics) (14). However, in left ventricular (LV) dysfunction, β-blockers may be poorly tolerated because of their marked negative inotropic effect. Thus a drug that would have a pure heart rate (HR)-reducing effect without a negative inotropic effect could be an interesting alternative to β-blockade.

Ivabradine (Iva) is a specific HR reduction (HRR) agent that inhibits the pacemaker hyperpolarization-activated current (If) in the sinus node (3), without any effect on inotropy (18, 25) and blood pressure (13). The beneficial effects of Iva have been shown during an early functional remodeling following myocardial infarction (MI). Indeed, a 3-mo treatment by Iva, started 7 days after MI in rats, increases stroke volume and preserves cardiac output despite the HRR (20). Recently, a 1-mo Iva treatment started 1 day after MI was shown to improve maximal myocardial perfusion and coronary reserve in rat hearts likely via the reduction of periarteriolar collagen (11). Because of the potential benefit that Iva treatment could bring to patients with a profoundly altered cardiac structure and function, it was important to study the effect of Iva-induced HRR in a situation of established severe congestive HF (CHF). We hypothesized that HRR in this pathological situation would have beneficial consequences on the cardiac neurohormonal drive and thus on cardiac collagen accumulation.

In this study, the Iva treatment was not started within the early post-MI, but 2 mo after MI and lasted 3 mo. We demonstrate for the first time in a post-MI chronic and severe HF that through its HRR effect, Iva markedly decreased ventricular excitability without harmful electrical consequences, prevented the worsening of LV dysfunction, and markedly decreased cardiac fibrosis and cardiac ACE and ANG II type 1 (AT1)-receptor transcripts, despite a late initiation after MI.

MATERIALS AND METHODS

Animals and treatments. Experiments were conducted according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Publication No. 85-23, Revised 1996) and had the agreement of the local Ethics Committee. Twelve-week-old male Wistar rats, weighing 220–240 g, were used. They had free access to a standard rat diet (Rats standard pellets, Ref. No 2016; Harlan). Rats were anesthetized by a ketamine (80 mg/kg)-xylazine (5 mg/kg) intraperitoneal mixture and mechanically ventilated, as described (24). MI was induced by a ligation of the left anterior descending coronary artery. Rats recovered from anesthesia in an aerated box heated at 37°C. The intervention led to a reproducible infarct involving ~30% of the LV circumference (24). The protocol was identical in sham-operated rats, but the ligature was not tied. Thirty percent of rats died from acute HF within 48 h after MI, and then 10% died every month.

One week after MI, a rapid echocardiography was performed to determine whether the MI was successful, and the size was quantified.
using planimetric measurement methods (Fig. 1). From our experience, rats whose ejection fraction (EF) was >40% at this time point would never become insufficient, and they were removed from the protocol.

Two months after MI, the cardiac dysfunction was quantified by echocardiography (Fig. 1). Rats with an EF < 30% were randomized in the MI and MI + Iva (10 mg·kg⁻¹·day⁻¹) homogeneous groups \((n \geq 12\text{/group})\). Iva as monohydrochloride salt \((S\,16257-2)\) was dissolved in drinking water. The treatment lasted for 3 mo. Five months after MI, a second echocardiography and 24-h ECG telemetry were performed (Fig. 1). Blood pressure was recorded under anesthesia using an intracarotid catheter. Euthanasia was then performed by an overdose of anesthetics. Excised hearts were rinsed in ice-cold 0.9% saline and blotted, and the atria and ventricles were separated and weighed. The ventricles were cut transversally into two parts. The basal part was included in Tissue-Tek (Sakura, Villeneuve d’Ascq, France) and frozen in liquid nitrogen-cooled isopentane for immunohistological studies. The scarred region and border zone were removed from the remaining apex part, which was frozen in liquid nitrogen-cooled isopentane for RNA and protein extraction. All samples were stored at \(-80^\circ\text{C}\) until use.

Twenty-four-hour telemetry monitoring. Twenty-four-hour telemetry was performed 1 day before the beginning of the treatment period as well as 1 day before the death of the animals. The ECG telemetry transmitter (DataScience International) was implanted in the peritoneum of anesthetized rats, with electrodes placed in a lead 2-like position. The ECG was recorded for 24 h. The acquisition, analysis, and automatic evaluation of mean RR, standard deviation of the RR (SDRR), PR, QRS and QT intervals and ventricular premature complexes (VPCs) were performed using ECG-Auto software (EMKA technologies). These measurements were randomly repeated for several diurnal and nocturnal periods of the 24-h telemetry recording. Furthermore, each sample was manually validated by two independent observers. A VPC was defined as an unexpected premature wide supraventricular QRS with a prematurity exceeding the normal variation of RR interval by 60% without a preceding P wave (26). A ventricular tachyarrhythmia was a regular wide QRS tachycardia with more than five consecutive VPCs according to the Lambeth Conventions (26).

Echocardiography. Transthoracic echocardiography was performed on anesthetized rats using an echocardiograph (General Electric Vivid 7; Paris, France), equipped with a 8–14-MHz linear transducer. Posterior end-diastolic and end-systolic LV posterior wall thicknesses and diameters were measured by the American Society of Echocardiography leading-edge method. LV end-diastolic (LVEDV) and end-systolic volumes were calculated using the area-length method. In addition, LV outflow velocity was measured by pulsed-wave Doppler, stroke volume, and cardiac output, calculated as cardiac output = aortic velocity-time integral \(\times [\pi \times (\text{LV outflow diameter/2})^2] \times \text{HR}\). The LVEF was measured using a modified version of Simpson's monoplane analysis (23). LV end-diastolic pressure (LVEDP) was calculated as previously described (16, 22).

Histological and morphometric analysis. An equatorial section of the heart was frozen in embedding medium. Cardiac cryosections (7 mm thick) were stained with picrosirisin red F3BA, and the interstitial collagen volume fraction was blindly quantified on five sections per heart (8–10 fields/section) as described (24). Pervascular collagen content was measured as described by Dedkov et al. (11). Briefly, it was defined as the ratio of the pervascular collagen area to the vessel area (media + lumen). For each heart (6–9 per group), 7 to 9 resistance vessels (50–200 μm in luminal diameter) per LV were evaluated.

Extraction of RNA and RT-quantitative PCR. Total RNAs from rat ventricles were extracted using RNeasy mini kit (Qiagen, Les Ulis, France) according to the manufacturer’s protocol with a DNase I treatment. The reverse transcriptions were carried out using the Ready-to-go kit (Amersham, Paris, France) and oligo-dT primer. Primers for rat ACE (GenBank accession no. AF532783) and rat AT1 receptor (GenBank no. M90065) were obtained using Primer-Express software (Applied Biosystems, Les Ulis, France). GAPDH (Taqman GAPDH rodent control reagent, Applied Biosystems) was selected as a housekeeping gene. PCR were carried out using Mastermix SYBr Green I (Applied Biosystems) according to the following protocol: 10 min of denaturation at 95°C, 30 s at 95°C, and 1 min at 60°C for 40 cycles (GeneAmp 5700, Applied Biosystems). Dissociation was carried out starting at 60°C. The \(2^{(-\Delta \text{Ct/1.1})}\) method (2), including the normalization to GAPDH, was applied for the quantification. The values of mRNA expression were expressed relative to a cardiac male rat RNA pool.

Western blot analysis. The abundance of ACE and AT1 proteins in the LV was determined by Western blot analysis, as described (1). Briefly, the samples were homogenized in a Triton X-100 lysis buffer, containing 1% Triton X-100 and (in mmol/l) 50 Tris-HCl (pH 7.4), 100 NaCl, 50 NaF, 5 EDTA, 0.1 leupeptin, and 0.001 aprotinin. After centrifugation (22,000 g, 15 min) at 4°C, the supernatants were collected and the proteins were quantified with a Qubit fluorometer (Invitrogen, Cergy-Pontoise, France). The proteins were separated by 4–12% acrylamide SDS-PAGE and electrotransferred onto nitrocellulose membranes using an blot (Invitrogen). After being stained with Ponceau S to ensure equal loading, the membranes were incubated overnight at 4°C with the antibodies directed against AT1 receptor (GenBank accession no. M90065) and ACE (dilution 1:100; MAB3502, Chemicon, Millipore, St. Quentin en Yvelines, France). An antibody against total actin (1:1,000, Sigma-Aldrich, St. Quentin-Fallavier, France) was used as a protein loading control. A chemiluminescent signal was produced using ECL loading control. A chemiluminescent signal was produced using ECL loading control. A chemiluminescent signal was produced using ECL (Invitrogen, Courbevoie, France). The proteins were separated by 4–12% acrylamide SDS-PAGE and electrotransferred onto nitrocellulose membranes using an blot (Invitrogen). After being stained with Ponceau S to ensure equal loading, the membranes were incubated overnight at 4°C with the antibodies directed against AT1 receptor (dilution 1:300; N-10, Santa Cruz Biotechnology, Santa Cruz, CA) and ACE (dilution 1:100; MAB3502, Chemicon, Millipore, St. Quentin en Yvelines, France). An antibody against total actin (1:1,000, Sigma-Aldrich, St. Quentin-Fallavier, France) was used as a protein loading control. A chemiluminescent signal was produced using ECL+ solution (Amercontrol, Orsay, France) and detected with a LAS-3000 luminescent image analyzer (FUJI, Courbevoie, France). The relative densitometry was determined using the computer software Multi Gauge V2.3 (FUJI).

Statistical analysis. Data are expressed as means ± SE. The difference between the control and Iva-treated groups at each phase of the study were assessed using two-way repeated-measures analysis of variance. When significant differences between groups were found, a pairwise multiple comparison procedure using Bonferroni t-tests were performed at each time point.

All tests were performed using StatView 5 software (Abacus). When indicated, a t-test was used for comparison between groups. \(P < 0.05\) was considered as significant.

RESULTS

Cardiac function. Two months after MI, i.e., at the time of randomization, echocardiography parameters confirmed that all MI rats had a severe HF with a significantly decreased LVEF (−58%) and an increased LVEDV (+64%; \(P < 0.001\)) and LVEDP (+220%; \(P < 0.001\)) compared with sham-operated rats (Table 1). Five months after MI, the cardiac...
Anatomical data and aortic pressure measured 5 mo after MI (Table 2).

Table 1. Echocardiographic parameters measured 2 and 5 mo after MI: before and after treatment

|                      | 2 mo After MI | 5 mo After MI (After 3 mo Treatment) | Treatment and Time Interaction (P Value)‡
|----------------------|--------------|-------------------------------------|--------------------------------------
|                      | Sham-operated | MI | MI + Iva | Sham-operated | MI | MI + Iva | MI | MI + Iva |
| n                    | 11           | 11 | 11       | 11           | 11 | 11       | 11 | 11       |
| Cycle length, ms     | 195±6        | 175±7* | 176±7*   | 194±7        | 174±6† | 201±15‡ | NS | <0.05    |
| LA, mm               | 0.41±0.01    | 0.56±0.02* | 0.56±0.02* | 0.41±0.01    | 0.62±0.01† | 0.55±0.02‡ | NS | <0.05    |
| LVEDV, ml            | 1.72±0.09*   | 1.75±0.08* | 1.75±0.08* | 0.83±0.01    | 2.21±0.08† | 1.77±0.02‡ | =0.02 | NS       |
| LVESV, ml            | 0.29±0.03    | 1.34±0.08* | 1.36±0.07* | 0.32±0.02    | 1.84±0.07† | 1.34±0.05‡ | =0.01 | NS       |
| Stroke volume, ml    | 0.54±0.03    | 0.39±0.05* | 0.39±0.07* | 0.52±0.03    | 0.37±0.03† | 0.42±0.04‡ | <0.05 | <0.05    |
| Cardiac output, ml/mn| 176±11       | 134±10*  | 133±11*   | 174±14       | 127±11† | 130±12 | NS | NS       |
| Ejection fraction, %  | 66±1         | 28±1*   | 29±1*     | 67±1         | 21±1† | 31±1‡ | <0.001 | NS       |
| LVEDP, mmHg          | 10±1         | 32±1*   | 32±1*     | 11±1         | 38±1† | 24±1‡ | =0.011  | <0.001   |

Values are means ± SE; n, number of rats. Iva, ivabradine; LA, left atrium; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEDP, left ventricular end-diastolic pressure; NS, not significant. *P < 0.01 and †P < 0.001 vs. sham-operated; ‡P < 0.01 vs. myocardial infarction (MI).

Table 2. Anatomical data and aortic pressure measured 5 mo after MI

<table>
<thead>
<tr>
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<th>Sham-Operated</th>
<th>MI</th>
<th>MI + Iva</th>
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<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Body Wt, g</td>
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<td>569±30</td>
<td>520±15*</td>
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<tr>
<td>Infarct size, %</td>
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<td>33±1</td>
<td>32±1</td>
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<td>Heart Wt, g</td>
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<td>LA + RA Wt, mg</td>
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<tr>
<td>LV + RV Wt, g</td>
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<td>LV + RV Wt/BW × 10³</td>
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<td>2.69±0.17</td>
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<td>Lungs Wt, g</td>
<td>2.1±0.06</td>
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<td>2.3±0.17*</td>
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<td>SAP, mmHg</td>
<td>169±6</td>
<td>133±11*</td>
<td>126±5*</td>
</tr>
<tr>
<td>DAP, mmHg</td>
<td>120±5</td>
<td>103±8</td>
<td>95±2*</td>
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Values are means ± SE; n, number of rats. LV, left ventricle; RV, right ventricle; RA, right atrium; SAP, systolic aortic pressure; DAP, diastolic aortic pressure. *P < 0.05 vs. sham-operated; †P < 0.05 vs. MI.

dysfunction of untreated MI rats worsened compared with the earlier values (ΔLVEF, −7%; ΔLVEDV, +0.49 ml; and ΔLVEDP, +6 mmHg) (Table 1). In addition, MI rats displayed clinical and anatomical signs of severe HF, namely, increased heart and lung weights (Table 2).

Conversely, in Iva-treated MI rats, all echocardiographic parameters revealed that the cardiac function impairment was stopped by treatment (Table 1). Hence, after 3 mo of treatment by Iva, LVEDV and LVESV were unchanged, whereas stroke volume and LVEDP were improved, compared with the pre-treatment values. The anatomical data of MI + Iva rats were not different from those of MI, except for the lung weight that was significantly decreased (Table 2). Interestingly, this study was not designed as a mortality study, we observed a decreased mortality rate in Iva-treated rats (23% vs. 50%; for MI + Iva and MI rats, respectively). Altogether, these results indicate an improvement of the clinical status of MI rats treated by Iva.

Twenty-four-hour telemetry parameters. HR was obtained from the telemetry-recorded ECG in vigil animals. No differences were observed between nocturnal and diurnal measurements. At baseline, 24-h telemetry showed a significant HR difference between sham-operated and MI rats (198 ± 7 vs. 170 ± 8 ms, respectively; P < 0.01). As shown in Fig. 2, the RR-interval cycle length was decreased by 9% in MI rats, as well as the HR variability time-domain parameter SDRR, compared with sham-operated rats (Fig. 2). In addition, an electrical remodeling was observed in these rats with increased PR, QRS, and QT intervals (Fig. 2).

After 3-mo treatment, whereas HR was unchanged in sham-operated and control MI rats (198 ± 7 vs. 197 ± 7 ms and 170 ± 8 vs. 173 ± 6 ms, respectively, between 2- and 5-mo periods), Iva increased the cycle length by 10.4% (P = 0.027)
as expected, improved SDRR (5 ± 1.5 vs. 3.9 ± 0.6 for MI; P < 0.05), and markedly decreased the VPC occurrence (514 ± 152 vs. 4,717 ± 1,363/day for MI; P = 0.023). On the other hand, PR, QRS, and QT intervals were not altered by Iva.

Ventricular perivascular and interstitial fibrosis. As already described (24), collagen abundance in the remote part of the LV was strongly increased in MI rats and involved both the perivascular and interstitial compartments (Figs. 3 and 4). As shown in Fig. 4A, interstitial collagen was increased by 402% (P < 0.001) in MI rats relative to sham-operated ones. After Iva treatment, interstitial collagen was reduced by 55% (P < 0.005) relative to MI rats, but the values did not reach the sham-operated ones. The perivascular fibrosis displayed a general scheme similar to that of interstitial fibrosis (Fig. 4B).

It was increased by 98% (P < 0.05) in the MI group relative to the sham-operated ones, but Iva did not significantly decrease it. Figure 5 shows that a linear regression was established between interstitial collagen volume fraction and LVEDP at 5 mo (r = 0.771; P < 0.001).

Renin-angiotensin-aldosterone system components gene expression. The ventricular gene and protein expressions of ACE and AT1 receptor were increased in MI rats (2.3- and 5.3-fold vs. sham-operated values, respectively, P < 0.05 for gene expression; and 1.95- and 1.78-fold vs. sham-operated values, respectively, P < 0.05 for protein expression) (Fig. 6). These increases were completely blunted by Iva treatment (P < 0.05).

DISCUSSION

This study shows for the first time that a late induction after MI of HRH by Iva treatment in rats with chronic and severe CHF 1) prevented the worsening of LV dysfunction and remodeling, 2) reduced ventricular excitability without harmful effects on electrical remodeling, 3) markedly decreased cardiac fibrosis, and 4) reduced the gene expression of renin-angiotensin system components.
fibrosis, 4) induced a downregulation of cardiac renin-angiotensin-aldosterone system (RAAS) transcripts, and 5) had no effect on systolic blood pressure.

Cardiac function. Mulder et al. (20) have first shown that Iva improved cardiac function and stroke volume in post-MI rats with LV dysfunction. However, Iva was started only 7 days post-MI before a marked decrease of the cardiac function. We report in our study that the post-MI cardiac dysfunction continued to worsen from 2 to 5 mo (Table 1). After the Iva treatment, all the functional indexes were similar to those initially measured, with, however, a significantly improved LVEDP and a trend toward a better LVEF (Table 1). It is noteworthy that these effects were observed even though Iva was started a long time after the MI. Cardiac function was thus preserved by Iva treatment as indicated by the marked reduction of lung weight. Therefore, the higher survival rate of the Iva-treated MI group might be related to the stabilization of several major cardiac functional parameters.

Iva and electrical remodeling. The hyperpolarization-activated cyclic nucleotides-gated underlying \( I_f \) is known to be a specific hyperpolarizing channel for sinus node action potential function (3). Hence, no effects on the effective refractory periods of atrial, atrioventricular node, and ventricular cells should be observed. Although in the setting of post-MI LV dysfunction these refractory periods are modified (increased dispersion and heterogeneity of refractory periods), we showed that \( I_f \) inhibition with Iva did not alter the duration of PR, QRS, and QT intervals in MI rats. In addition, no increasing VPCs or sustained and nonsustained ventricular arrhythmias were observed in Iva-treated MI rats. Altogether, these results indicated that Iva did not bear proarrhythmogenic effects in the setting of post-MI-modified electrophysiological conditions.

The parameters of HR variability have shown their superiority over the usual invasive and noninvasive parameters in predicting sudden death in patients with low EF post-MI (6). The decrease in the mean values of the RR interval and the SDRR is positively correlated with the occurrence of sudden cardiac death and death of any origin (15). Furthermore, HR variability-time and frequency-domain parameters are considered as markers of the autonomous nervous system status (24a). In our experimental study, we found that Iva treatment significantly improved time-domain parameter SDRR and reduced HR, indicating a beneficial effect on the autonomous nervous system balance with a shift toward vagal tone. This could be related to two factors: an improvement of LV function leading to a reduction of the sympathetic drive and/or to a decreased RAAS stimulation.

Ventricular tachyarrhythmias following MI are known to occur when ventricular remodeling (ventricular dilation and fibrosis) is associated with an imbalance of the autonomic nervous system with sympathetic activation and the presence of a trigger, i.e., ventricular premature complexes (7, 12). As already discussed, Iva had a marked positive effect on these parameters. We showed that Iva treatment obviously reduced VPCs, in addition to the decreased ventricular fibrosis and sympathetic drive. Altogether, these results indicated a poten-

Fig. 6. Expression of ventricular renin-angiotensin-aldosterone system components. The mRNAs and proteins were prepared from the remote zone of left ventricular tissues. The abundance of mRNAs was measured by real-time PCR and normalized by GAPDH, and the proteins were quantified by Western blot analysis as described in MATERIALS AND METHODS. Values were normalized to the mean of sham-operated values and expressed as arbitrary units (AU).

Fig. 5. Relationship between ventricular fibrosis and left ventricular end-diastolic pressure (LVEDP) in MI rats. Linear regression between left ventricular interstitial collagen volume fraction and LVEDP (n = 23; \( R = -0.77; P < 0.001 \)).
tional benefit on the ventricular arrhythmogenic substrate that could lead to a reduction of both cardiac and arrhythmic deaths in HF patients, although further studies are mandatory to confirm these hypotheses.

**Cardiac fibrosis and RAAS.** MI induces important cardiac remodeling, including fibrosis of the remote myocardium and myocyte hypertrophy (21). One remarkable result is the decrease of ventricular interstitial fibrosis that was observed in Iva + MI rats compared with MI rats. Previously, Iva was shown to prevent the collagen accumulation in the post-MI ventricle (11, 20). However, this prevention was observed while Iva was initiated at 7 days post-MI. The major finding herein is that Iva was able to decrease and/or stop the accumulation of collagen in the LV remote part even when Iva was started long ago from the MI, a decreased fibrosis known to be beneficial on cardiac function (27). This is a significant advance for the potential use of this drug in clinical practice, since we may anticipate that numerous patients with HF will have an already-established cardiac fibrosis, which continues to expand. To investigate whether this curative beneficial effect of Iva on fibrosis was related to the level of cardiac RAAS stimulation, we quantified the transcript level of the two main RAAS genes that are evidenced in adult heart (9). Cardiac ACE expression is a marker of the activation of cardiac RAAS (8), and AT$_1$ receptor is the main ANG II receptor in the heart. The decreased levels of ACE and AT$_1$ receptor mRNA and proteins observed in Iva + MI strongly suggested that the low level of RAAS activation in response to treatment prevented the fibrotic remodeling of the remote myocardium.

**Mechanisms of Iva efficacy on cardiac function, electrical remodeling, and fibrosis.** The mechanisms by which HRR per se improves cardiac function have been studied, both at rest and during exercise. HR is a major determinant of the balance between myocardial oxygen supply and demand. The Iva-induced HRR prolongs diastole and in consequence increases the myocardial oxygen supply in dogs (5). On the other hand, oxygen demand is reduced with HRR, improving the oxygen supply-to-demand ratio (18). Moreover, with the use of β$_1$-selective blockade, a chronic reduction of HR promotes coronary angiogenesis and, thereby, facilitates regional myocardial perfusion after MI (10). Thus Iva displays anti-ischemic properties, which is the rationale for an indication in patients with coronary artery disease (17). This study points to a new mechanism that likely participates in a stabilization of cardiac function and a degradation prevention in HF. It might be proposed that HRR enhanced oxygen delivery to the myocardium by an increase in regional myocardial perfusion. Myocardial oxygen consumption was also decreased by bradycardia, resulting in an improvement in energetic balance. The latter led to an improvement of ventricular function with better cardiac hemodynamics resulting in a decreased neurohormonal stimulation. Reductions in sympathetic drive and in the imbalance of sympathetic/vagal tone underlie the improvement of the time-domain HR variability parameter and the threshold level for ventricular arrhythmias occurrence. At the same time, a reduction of RAAS activation led to an improvement of renal and vascular pressures that decreased the cardiac wall stress resulting in the prevention of the worsening of cardiac remodeling as well as the occurrence of cardiac fibrosis and stretch-induced ventricular excitability. Thus, through its negative chronotropic effects, Iva allowed the maintenance of cardiac function below the threshold for both RAAS and sympathetic tone stimulation, even in a severe post-MI HF setting.

**Limitations.** A limitation of our model is the absence of sustained ventricular tachyarrhythmias, which are considered to be predictors of sudden cardiac death (19). Hence, a diminution of these arrhythmias by Iva treatment could have underlined some antiarrhythmic properties of Iva. However, the marked decrease of VPCs in addition to the reduction of ventricular fibrosis and function impairment as well as sympathetic drive may indicate a protective effect of Iva on these well-known arrhythmogenic factors. In addition, although we did not aim to assess the mortality in our study, we observed an improvement in the survival rate in Iva-treated rats correlated to a better cardiac hemodynamic that may be related to a decrease of arrhythmic and cardiac deaths. One may, however, note that the lower mortality rate in the Iva group may have led to an underestimation of the beneficial effects of Iva treatment, with the less severe HF rats being left in the control group.

**Conclusion.** This study shows that HRR by Iva treatment of rats with MI and severe CHF, despite a late initiation after MI, prevented anatomical, tissular, and electrical remodeling. This efficacy in preventing the worsening of LV dysfunction and remodeling may be related to a downregulation of cardiac RAAS transcripts. Such beneficial effects of Iva on cardiac remodeling open new clinical perspectives for the treatment of severe HF.

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