Effects of long-term angiotensin II AT<sub>1</sub> receptor blockade on survival, hemodynamics and cardiac remodeling in chronic heart failure in rats<sup>1</sup>

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Abstract

Objective: The beneficial effect of chronic angiotensin I converting enzyme (ACE) inhibition on survival has for long been established in the rat post-infarction model of chronic heart failure (CHF) and has subsequently been confirmed in humans. This study investigates in rats whether chronic angiotensin II AT<sub>1</sub> receptor blockade shares with ACE inhibition the same beneficial effect. Methods: Rats were subjected to coronary artery ligation and, from 7 days later, orally treated for 7.5 months with placebo or irbesartan (5 or 50 mg/kg/day). Results: Irbesartan dose-dependently increased survival (placebo: 27%, low dose: 52%, high dose: 82%, sham-ligated: 100%; high dose vs placebo: P<0.001 and vs low dose: P<0.05; low dose vs placebo: P=0.11). Irbesartan also dose-dependently decreased urinary cyclic GMP excretion throughout the study. At 7.5 months, it dose-dependently decreased left ventricular (LV) end diastolic pressure, normalized LV pressure maximal rate of rise (dP/dt) and cardiac index values and improved LV and right ventricular regional blood flows (radioactive microspheres) and resistances. At 7.5 months, irbesartan markedly decreased myocardial hypertrophy but had almost no effect on LV dilatation and subendocardial fibrosis. Conclusions: Long-term angiotensin II AT<sub>1</sub> receptor blockade with irbesartan strongly and dose-dependently increases survival in the rat model of coronary ligation-induced CHF. This effect is due to the combination of the beneficial effects that the drug exerts on systemic and coronary hemodynamics, on cardiac pump function and vs cardiac hypertrophy development. Long-term AT<sub>1</sub> receptor blockade might thus prove useful and prolong survival in human CHF. © 1999 Elsevier Science BV. All rights reserved.

Keywords: Experimental heart failure; Survival; Angiotensin II AT<sub>1</sub> receptor blockade; Hemodynamics; Cardiac remodeling; Rat

1. Introduction

Angiotensin I-converting enzyme (ACE) inhibition is now an established therapy for patients with chronic heart failure (CHF) and systolic left ventricular (LV) dysfunction and for patients who have had a myocardial infarction [1–5]. In these patients, ACE inhibitors reduce morbidity and mortality and improve the quality of life.

The beneficial effects of chronic ACE inhibition on survival were initially described with captopril in the rat model of coronary ligation-induced myocardial infarction [6]. In subsequent studies, these beneficial effects were confirmed with other ACE inhibitors [7–10] and their mechanisms investigated. These include improvement of the hemodynamic status [8–12] with reduced afterload and preload, decreased sympathetic activity [13] and limitation or prevention of cardiac and vascular remodeling [8–10,14]. These effects have mostly been attributed to blockade of angiotensin II production and/or to a decrease in the breakdown of bradykinin. However, whereas bradykinin accumulation may contribute through the release of nitric oxide and prostacyclin to the beneficial hemodynamic and structural effects of ACE inhibitors, it is also thought that it could be responsible for a number of adverse reactions observed in humans, e.g., cough, angioedema, renal dysfunction, etc. Moreover, it has recently

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been postulated that bradykinin was responsible for the increased mortality observed after ACE inhibition during acute myocardial infarction in rats [15].

Non-peptidic angiotensin II type I receptor antagonists (AT₁ blockers) block these receptors specifically without increasing bradykinin levels [16] although an interaction with other autacoids cannot be excluded [17]. Moreover, as angiotensin II may be produced by ACE-independent pathways [18], AT₁ blockers could be more active than ACE inhibitors at fully interrupting the renin-angiotensin system. Therefore, they might offer efficacy and safety advantages over ACE inhibitors in the treatment of CHF. And indeed, oral losartan has been found to exert the same beneficial hemodynamic effects as ACE inhibitors in patients with symptomatic heart failure [19], and in elderly CHF patients it was found to be associated with a lower mortality and a better tolerability than captopril [20]. In the latter study, however, the reduction in mortality observed with losartan was primarily due to a decrease in all-cause mortality and especially in the number of sudden deaths.

More recently, another study (Resolv) [21] comparing the effects of candesartan, enalapril and their combination in patients with CHF was interrupted two months before completion due to concerns about increased mortality and morbidity events with the candesartan monotherapy and the candesartan/enalapril combination vs the enalapril monotherapy. Therefore, the issue of the beneficial effects of AT₁ receptor blockade on mortality in patients with CHF is not definitely settled to date.

Experimental investigations of AT₁ blockers effects on survival are very scarce. One one-year study performed in the rat model of post myocardial infarction CHF found no difference between losartan and captopril regarding survival [22] but, as there was no control group in that study, this was clearly not a demonstration that long-term AT₁ receptor blockade prolongs survival in this experimental model. We therefore decided to readdress the issue of the potential beneficial effects of chronic AT₁ blockers administration on survival in the rat model of post-infarction CHF using irbesartan, a newly developed AT₁ blocker [23,24], at two doses in a double controlled (untreated sham and untreated infarcted rats) study. The treatment was started 7 days after coronary artery ligation, i.e., when the inflammatory process of infarction was over and when scar formation was almost complete [6]. In addition, and in order to determine the mechanisms involved in the treatment-induced effects, we also investigated a number of hemodynamic, biological and cardiac morphological parameters.

2. Methods

2.1. Animals and treatments

All experiments were performed in accordance with the official regulations edicted by the French Ministry of Agriculture and conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Ten week-old male Wistar rats (Iffa Credo, L’Arbresle, France) were subjected to ligation of the left coronary artery as previously described [25]. Briefly, the rats were anesthetized with ether, intubated and ventilated. A left thoracotomy was then performed, the heart was exteriorized and the left coronary artery was ligated 2 mm from its origin. Subsequently, the chest was closed and the rats were allowed to recover. Seven days after surgery (23 animals died during that week), an electrocardiogram was recorded under ether anesthesia in all surviving animals, and those (n=14) with no electrical signs of myocardial infarction (MI) were discarded. The remaining rats with MI (n=89) were randomly assigned to one of the three following groups: MI-controls (n=30), MI-Irbesartan, 5 mg/kg (MI-Ir5, n=30) and MI-Irbesartan, 50 mg/kg (MI-Ir50, n=29). For estimation of groups size, it was assumed from the literature [5,6,8,10] that the mortality rate at 9 months (the scheduled duration of our study) in MI-controls is approximately 80%. Considering that the aim was to decrease this percentage down to at least 30%, the required number of animals (risk α: 5%, risk β: 5%) was 28 per group.

Finally, 29 ten week-old male Wistar rats (Iffa Credo, L’Arbresle, France) underwent the same surgical procedure as described above but ligation of the left coronary artery was not performed. These animals formed the Sham group (S, n=29).

Animals from all groups were housed 5 per cage, fed ad libitum with standard diet (AO4, 0.130 mEq Na⁺/g, 0.205 mEq K⁺/g, UAR, Epinay-sur-Orge, France) and had free access to tap water. Room temperature was maintained at 21±1°C and a 12 h light/dark cycle was programmed.

Starting from the 8th post-ligation day, animals from groups MI-Ir5 and MI-Ir50 were given irbesartan, incorporated in their food, at the daily doses of 5 (MI-Ir5) or 50 (MI-Ir50) mg/kg until the end of the study. In order to take into account the evolutions with age of the animals’ body weight and food intake, these two parameters were measured weekly, and different batches of chow (UAR, Epinay-sur-Orge, France) containing increasing concentrations of irbesartan were used successively in order to ensure that the animals received the appropriate daily dose of irbesartan throughout the study. This goal was achieved as, at the end of the study, the calculated mean±s.e.mean doses of irbesartan that had been administered to the two treated groups were 4.9±0.1 (MI-Ir5) and 49.9±0.6 (MI-Ir50) mg/kg, respectively. The daily oral doses of 5 and 50 mg/kg of irbesartan were chosen in a preliminary experiment as those reducing after 6 weeks of treatment the increments in mean arterial pressure induced by angiotensin II (in the range of 0.1 to 1 μg/kg, i.v.) by
40-50 and 75-85%, respectively, in the pithed normoten-
sive Wistar rat.

2.2. Survival

Initially, the survival study was programmed to last 9 months and at its term a hemodynamic study was scheduled. However, as it turned out that the mortality was somewhat greater than expected in MI-controls, the survival study was interrupted after 235 days (hereafter referred to as 7.5 months) in order to allow the determination of the hemodynamic parameters in a sufficient number of MI-
controls.

During the 7.5 months period of the study, cages were inspected daily for deceased animals in order to calculate survival rate. Hearts of deceased animals were removed and fixed in Bouin’s solution for subsequent determination of infarct size.

2.3. Hemodynamic parameters

Systolic arterial pressure (SAP) (photoelectric pulse detector PC 139, IITC, Woodland Hills, CA, USA) and heart rate (HR) were measured in the restrained conscious rat in all surviving animals after 1, 3, 6 and 7.5 months of treatment. Simultaneously, body weight (BW) was also determined.

At the end of the study (7.5 months), 14 randomly selected animals from the S group and all the surviving animals from the MI-controls, MI-Ir5 and MI-Ir50 groups were anesthetized (sodium pentobarbitone, 50 mg/kg, i.p.) and prepared for measurement of cardiac output and regional blood flows by the radioactive microspheres technique [26]. The right carotid artery was catheterized (0.60 mm I D, PE tubing) for blood pressure measurement (Statham P10EZ transducer, Gould amplifier 13-4615-10, Gould Instruments, Cleveland, OH, USA) and for microspheres injection, as well as the right femoral artery (0.28 mm I D, PE tubing) for collection of the reference blood sample. After blood pressure stabilization, the tip of the right carotid artery was gently advanced into the LV cavity for the recording of LV pressure, LV pressure maximal rate of rise (dP/dr) (Gould differen- tiator 13-4615-71, Gould Instruments, Cleveland, OH, USA) and LV end diastolic pressure (LVEDP) (Gould Instruments, Cleveland, OH, USA). Radioactive microspheres (141Ce, diameter: 15±3 μm, specific activity: 7.59 mCi/g, NEN Company, Bos-
ton, MA, USA) were diluted 1:10 in a 20% dextran solution and sonicated for 10 min. Then, 0.2 ml of this dilution (i.e., approximately 60000 to 80000 beads) were injected in 10 s into the LV followed by 0.2 ml saline. The radioactivity of the injection syringe was counted (Com-
pugamma LKB 1280, Turku, Finland), before and after injection, enabling the total radioactivity injected (TR) to be determined from the difference. The reference blood sample was taken from the right femoral artery and collected in a previously heparinized and weighed syringe at a flow rate of 0.7 ml/min (Harvard 907A pump, Harvard, Southnatick, MA, USA) for 90 s, the sampling beginning 10 s before the microspheres injection. The radioactivity of this reference blood sample (Rf) was determined.

When hemodynamic studies were completed, a 2 ml blood sample (for angiotensin II plasma levels determination) was withdrawn from the abdominal aorta into tubes containing Na2EDTA and centrifuged, and the plasma was frozen and stored at −80°C. Thereafter, the animals were sacrificed and the heart (left and right ventricles) and kidneys were removed from each animal, cleaned, dried on filter paper, weighed fresh and counted. Finally, left and right ventricles were processed for subsequent histological analysis.

The reference flow Qf was calculated as:

\[ Q_f = \frac{R_b \times C \times R_{out} \times C_{out}}{R_{out} \times C_{out} \times R_{in} \times C_{in} \times R_{in} \times C_{in}} \]

where:

- Rb: reference blood sample weight × 60/sampling time (90 s) × blood density (1.06),
- C: cardiac output as: cardiac radioactivity × cardiac output/organ weight × TR,
- Rf, total peripheral resistance as: mean arterial pressure (MAP)/ cardiac index, and individual organ vascular resistance as: MAP/individual organ blood flow.

2.4. Cardiac morphological parameters

After sacrifice at 7.5 months, heart weight (HW) and right ventricular weight (RVW) were measured and expressed as HW/BW and RVW/BW values.

Left ventricles were then immersed in Bouin’s fixative solution. After fixation, the ventricles were cut into 4 transversal slices (1 basal, 2 mid and 1 apical, 2 mm thick each) which were dehydrated (graded ethanol solutions) and embedded in paraffin. One 3 μm thick section was obtained from each slice and stained with Sirius red.

The whole histomorphometric study was performed using a computer-assisted image analyzer (NS 15000, Microvision, Evry, France) as previously described [8,9]. For infarct size measurement, the two mid ventricular slices were examined using a monozoom (mean magnification: ×4) camera connected to the image analyzer. The image transmitted from the camera to the analyzer was digitized in 512X512 pixels with 256 levels of grey. Infarct size (mean of the two slices determinations, %) was expressed as the ratio of the sum of external and internal scar lengths to the sum of external and internal diameters of the left ventricle. These scar lengths and LV perimeters were directly determined from the two mid ventricular slices (mean of the two slices) (monozoom: ×4). Finally, LV dilatation was estimated as the LV internal perimeter (mm).

For collagen density network, the image analyzer was connected to a light microscope. Each slide was examined at 250× magnification. Each field was digitized at 512× 512 pixels with 256 levels of grey. Twenty fields were analyzed in the endocardium of the viable LV wall.
Collagen density (mean of the 20 determinations) was expressed as the ratio of collagen area to myocardial area (%). This index takes into account both interstitial (reactive) and scar (reparative) fibrosis, but not perivascular fibrosis.

2.5. Biological parameters

Urinary cyclic GMP (radioimmunoassay, Amersham, Little Chalfont, UK), diuresis and creatininuria were measured on 16 h urine samples taken from 10 to 15 randomly selected animals from each group at 1, 3, 6 and 7.5 months, and placed in metabolic cages. Urinary cyclic GMP was normalized to urinary creatinine levels.

Angiotensin II plasma levels were measured by radioimmunoassay (Eria Diagnostics Pasteur, Marnes la Coquette, France) after prior angiotensin II extraction carried out on Bond-Elut (phenyl-PH minicolumns) using methanol. The detection limit was 0.95 pg/ml of angiotensin II. Intraassay and interassay variation coefficients were 9.8 and 14.4%, respectively.

2.6. Drug

Irbesartan, or 2-n-butyl-4-spirocyclopentane-1-[2'- (tetrazol-5-yl) biphenyl-4-yl) methyl]-2-imidazolin-5-one, was supplied by Sanoﬁ Recherche (Montpellier, France).

2.7. Statistical analysis

All results, except survival, are expressed as means± s.e. mean.

Comparison of survival in the four groups of animals was performed using the method described by Mantel [27]. The relative risk of death (RRD) between each pair of groups was calculated. For instance, for groups MI-Ir5 and MI-controls, the RRD is the ratio of ($O_{MI-Ir5}/E_{MI-Ir5}$) to ($O_{MI-controls}/E_{MI-controls}$), where $O_{MI-Ir5}$ and $O_{MI-controls}$ are the observed and $E_{MI-Ir5}$ and $E_{MI-controls}$ the estimated numbers of deaths, respectively, in these two groups, assuming the equality of the survivor functions across both groups [27].

For all parameters, statistical analysis was performed at each evaluation time using a one-way ANOVA confirmed by a non parametric test (Kruskal–Wallis). In case of global significance ($P<0.05$), a Bonferroni’s correction test was performed for comparisons between the different groups.

3. Results

3.1. Infarct size

At the end of the study, the hearts of the 89 MI-rats included in the study, whether spontaneously deceased or sacrificed at 7.5 months, were examined for infarct size. Two animals, one from group MI-Ir5 (infarct size: 15.6%) and one from group MI-Ir50 (infarct size: 18.1%), had an infarct size > 20% of total LV and were thus excluded from the study. Final sizes of the groups were thus 30, 29, 28 and 29 for groups MI-controls, MI-Ir5, MI-Ir50 and S, respectively.

Infarct size estimated at the end of the study on all animals, whether spontaneously deceased or sacrificed, was 40.6±1.1, 38.6±1.5 and 38.5±1.0 in groups MI-controls, MI-Ir5 and MI-Ir50, respectively. These values were not statistically different from each other.

Among spontaneously deceased animals, the figures were 42.1±0.9, 44.2±1.4 and 43.7±1.6, there being no significant difference between these values. As evidenced in Fig. 1 which plots infarct size values vs survival duration, there was no correlation between these parameters either in MI-controls ($r=0.153$, NS) or in MI-Ir5 ($r=0.305$, NS) and MI-Ir50 ($r=0.157$, NS) groups.

Among animals sacrificed at 7.5 months, mean infarct size values were 36.4±2.8, 33.5±1.6 and 37.4±1.0%, respectively, the values in group MI-Ir50 (37.4±1.0%) being significantly greater ($P<0.05$) than in group MI-Ir5 (33.5±1.6%).

3.2. Survival

No animal died in the S group during the 7.5 months duration of the study.

Fig. 2 illustrates the survival curves in the three groups of MI-rats. At the end of the study, 22 (out of 30, i.e., 73%), 14 (out of 29, i.e., 48%) and 5 animals (out of 28, i.e., 18%) had died spontaneously in MI-controls, MI-Ir5 and MI-Ir50 groups, respectively. Median survival time was 184 days in MI-controls. As shown on Fig. 2, irbesartan, 50 mg/kg, markedly increased survival as compared to MI-controls ($P<0.001$), an effect that was already significant at 6 months ($P<0.01$). Irbesartan, 5 mg/kg, also showed a trend to increase survival at 7.5 months, but this effect did not reach statistical significance.
Finally, survival in the MI-Ir50 group was significantly greater than in the MI-Ir5 group (P<0.05).

Relative risk of death values calculated over the 7.5 months study period were 0.577 (MI-Ir5 vs MI-controls), 0.162 (MI-Ir50 vs MI-controls) and 0.297 (MI-Ir50 vs MI-Ir5), indicating that the risk of death was reduced by approximately 6 fold by irbesartan, 50 mg/kg (vs MI-controls), by 2 fold by irbesartan, 5 mg/kg (vs MI-controls) and by 3 fold by irbesartan, 50 mg/kg vs irbesartan, 5 mg/kg.

### 3.3. Hemodynamic parameters

Table 1 shows SAP values as measured in the conscious state in the four groups of animals at the end of the study. SAP of MI-rats remained significantly lower than in S-rats throughout the study (data not shown), and irbesartan, whichever the dose, was almost neutral on SAP as compared to MI-controls. Heart rate was and remained similar in the four groups throughout the study (data not shown).

Table 2 shows MAP, heart rate, cardiac index, total peripheral resistance, dP/dt and LVEDP values as measured in the four groups of anesthetized animals at 7.5 months. As can be seen, myocardial infarction (group MI-controls vs group S) decreased MAP, heart rate, dP/dt and cardiac index but, given the small number of animals in each group (n=6 and 14 in groups MI-controls and S, respectively), these changes were not significant. In contrast, myocardial infarction resulted in a strong increase in LVEDP (+392%, P<0.05). Irbesartan at both doses improved the hemodynamic status, increasing (as com-
Body weight and cardiac morphological parameters

Body weight was similar throughout the study in S, MI-controls and MI-Ir5 groups (data not shown). It was, however, significantly smaller in the MI-Ir50 group than in the three other groups throughout the study, e.g., −7, −12, −11 and −9% vs MI-controls at 1, 3, 6 and 7.5 months, respectively (Table 1). This occurred despite similar food consumption values in the four groups of animals (21.9±0.5, 22.1±0.3, 22.9±0.3 and 21.6±0.3 g/24 h per rat in S-rats, MI-controls, MI-Ir5 and MI-Ir50, respectively).

Values of the investigated cardiac morphological parameters, as measured in the animals of the four groups that survived and were sacrificed at 7.5 months, are shown in Table 1. As expected, myocardial infarction resulted in significant increases in HW, HW/BW and RVW/BW, LV internal perimeter and LV collagen density (MI-controls vs S-rats). Irbesartan at both doses tended to reduce HW (−16%, NS, and −27%, P<0.05, vs MI-controls), HW/BW (−16 and −15%) and RVW/BW (−21 and −20%). In contrast, LV internal perimeter (−9 and −8% vs MI-controls, NS) and LV collagen density (−15 and −2% vs MI-controls, NS) were almost not modified by irbesartan, 5 and 50 mg/kg.

3.5. Biological parameters

In MI-controls, urinary cyclic GMP was strongly increased as compared to S-rats at 1 month and, although decreasing with age, remained significantly greater than in S-rats throughout the study (Fig. 3). In the MI-Ir5 and, to a larger extent, in the MI-Ir50 groups, urinary cyclic GMP was smaller than in MI-controls throughout the study and often significantly so (Fig. 3). Diuresis and creatininuria were and remained similar in the four groups of animals throughout the study (data not shown).

Angiotensin II plasma levels measured at 7.5 months in groups S, MI-controls, MI-Ir5 and MI-Ir50 were 169±4, 207±27 (NS vs S-rats), 590±160 (P<0.05 vs S-rats, NS vs S-rats), 174±15, 174±12, 169±9, 169±9, respectively. MAP: mean arterial pressure, HR: heart rate, CI: cardiac index, TPR: total peripheral resistance, LVEDP: left ventricular end diastolic pressure, LV: left ventricle, RV: right ventricle. Values are mean±s.e.mean. *P<0.05 vs corresponding S-rats value, †P<0.05 vs corresponding MI-controls value.

Table 2
Values of the hemodynamic parameters (measured in the anesthetized animals at 7.5 months) in the four groups of animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S-Rats</th>
<th>MI-Controls</th>
<th>MI-Ir5</th>
<th>MI-Ir50</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>122±4</td>
<td>102±13</td>
<td>117±4</td>
<td>104±8</td>
</tr>
<tr>
<td>HR (beats.min⁻¹)</td>
<td>350±8</td>
<td>314±17</td>
<td>342±10</td>
<td>353±12</td>
</tr>
<tr>
<td>CI (ml.min⁻¹.kg⁻¹)</td>
<td>160±8</td>
<td>146±15</td>
<td>174±12</td>
<td>169±9</td>
</tr>
<tr>
<td>TPR (mmHg.ml⁻¹.min.kg⁻¹)</td>
<td>0.79±0.04</td>
<td>0.74±0.14</td>
<td>0.70±0.05</td>
<td>0.63±0.06</td>
</tr>
<tr>
<td>dP/dt (mmHg.s⁻¹)</td>
<td>6207±478</td>
<td>4542±797</td>
<td>5788±625</td>
<td>5450±494</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.8±1.5</td>
<td>18.7±7.6*</td>
<td>15.2±3.8*</td>
<td>6.8±2.0*</td>
</tr>
<tr>
<td>LV Flow (ml.min⁻¹.g⁻¹)</td>
<td>3.35±0.29</td>
<td>1.89±0.28*</td>
<td>2.99±0.32</td>
<td>2.45±0.17</td>
</tr>
<tr>
<td>RV Flow (ml.min⁻¹.g⁻¹)</td>
<td>3.83±0.34</td>
<td>2.45±0.34</td>
<td>4.11±0.51</td>
<td>3.31±0.40</td>
</tr>
<tr>
<td>Renal Flow (ml.min⁻¹.g⁻¹)</td>
<td>5.01±0.35</td>
<td>5.19±0.77</td>
<td>6.38±0.51</td>
<td>5.17±0.31</td>
</tr>
<tr>
<td>Renal Resistance (mmHg.ml⁻¹.min.g⁻¹)</td>
<td>26.2±2.2</td>
<td>24.0±6.4</td>
<td>20.3±2.5</td>
<td>21.5±2.7</td>
</tr>
</tbody>
</table>

S-rats (n=14): sham-operated rats, MI-controls (n=6): control infarcted rats, MI-Ir5 (n=13) and MI-Ir50 (n=10): infarcted rats treated for 7.5 months with irbesartan, 5 and 50 mg/kg/day, respectively. MAP: mean arterial pressure, HR: heart rate, CI: cardiac index, TPR: total peripheral resistance, LVEDP: left ventricular end diastolic pressure, LV: left ventricle, RV: right ventricle. Values are mean±s.e.mean. *P<0.05 vs corresponding S-rats value, †P<0.05 vs corresponding MI-controls value.

Fig. 3. Mean (± SEM) values of urinary cyclic GMP (nmol/µmol of creatinine) measured at 1, 3, 6 and 7.5 months in conscious sham-operated rats (●), control infarcted rats (MI-controls, ■) and infarcted rats treated for 7.5 months with irbesartan, 5 mg/kg/day (MI-Ir5, □) or 50 mg/kg/day (MI-Ir50, △). *P<0.05 vs corresponding value in sham-operated rats. †P<0.05 or better vs corresponding value in MI-controls.
vs MI-controls) and 869±174 pg/ml (P<0.05 vs S-rats and MI-controls, NS vs MI-Ir5), respectively.

4. Discussion

The main result of this study is that the angiotensin II AT1 receptor antagonist, irbesartan, strongly and dose-dependently prolonged long-term survival in the rat post-infarction model of CHF. Irbesartan also tended after 7.5 months of treatment to improve the hemodynamic status of the animals and to limit the development of cardiac hypertrophy.

Numerous studies have previously demonstrated that long-term administration of ACE inhibitors does prolong survival in the rat post-ischemic model of CHF [6–10] and these data have later on been confirmed in patients [1–5]. Regarding AT1 blockers, only one study comparing the effects of losartan and captopril administered for one year to post-ischemic CHF rats has been reported [22] which showed that the survival curves obtained with the two drugs did not differ significantly from each other. However, as there was no control group in that study, and despite the fact that captopril has previously been shown to significantly reduce mortality in this model [6], the data reported by Milavetz et al. [22] cannot be considered as a demonstration of the efficacy of AT1 receptor blockade at prolonging survival in CHF rats. In the present study, three groups of infarcted animals were investigated: one untreated group (MI-controls), one treated with a low (5 mg/kg/day) and one treated with a high (50 mg/kg/day) dose of irbesartan. Mean infarct size was similar in these three groups of animals (40.6±1.1, 38.6±1.5 and 38.5±1.0%, respectively, NS). An additional group of non-infarcted (sham ligation, S) rats was also included in the study. Our data clearly show that irbesartan dose-dependently reduced death occurrence, the survival curve obtained in the MI-Ir50 group being, over the 7.5 months observation period, significantly different not only from that of the MI-controls (P<0.001) but also from that of the MI-Ir5 group (P<0.05). Regarding the latter survival curve, it was not significantly different from that of MI-controls (P=0.11) but, given the slopes of the two curves, it may reasonably be assumed that if the study had lasted 9 months as initially scheduled and if the size of the experimental groups had been greater, the difference between them would have reached statistical significance. Anyhow, the relative risk of death was reduced 2 fold with the low dose and 6 fold with the high dose of irbesartan, and mortality was reduced 3 fold with the vs the low dose of irbesartan. Finally, the dose-dependency of irbesartan’s beneficial effects in this model is also apparent (a) from the kinetics of these effects as the MI-Ir50 survival curve diverges from that of MI-controls 6 weeks before the same phenomenon is observed for the MI-Ir5 survival curve, and (b) from the dose-related decreases in urinary cyclic GMP, a specific marker of the severity of CHF in this rat model [28], observed throughout the study in the irbesartan-treated MI-rats as compared to MI-controls. To our knowledge, these results taken all together are the first demonstration that (a) chronic AT1 receptor blockade shares with chronic ACE inhibition the ability to reduce morbidity and to prolong survival in the rat model of post-infarction CHF, thereby confirming that interruption of the renin-angiotensin system is of paramount importance in these beneficial effects, and (b) the latter are, at least with irbesartan, dose-dependent in intensity and rapidity of onset. Given the results of a preliminary study which showed that angiotensin II (0.1 to 1 µg/kg, i.v.)-induced increments in mean arterial pressure in the pithed rat were decreased by 40–50 and 75–85% by irbesartan, 5 and 50 mg/kg/day for 6 weeks, respectively (see Section 2), it thus appears that the beneficial effects of irbesartan in this study directly parallel the intensity of the drug-induced AT1 receptor blockade and the resulting dose-dependent increase in angiotensin II plasma levels.

It could be considered that the doses of irbesartan administered in the present study (5 and 50 mg/kg/day) are high doses when compared to those used in humans. However, the same remark also applies to all other drugs investigated and found to be effective in the same rat model of CHF (e.g., 200 mg/kg/day for captopril [6], 30 mg/kg/day for lisinopril [10], 2.5 mg/kg/day for enalapril [7], 200 mg/kg/day for losartan [22], 15 and 10 mg/kg/day for mibefradil and amlodipine, respectively [29], etc.) which probably corresponds to a species characteristic.

In the MI-controls taken as a whole (spontaneously deceased and sacrificed animals), mean infarct size was 40.6±1.1%. Among the thirty animals of the group, 22 (73%) had a large infarct (>40%) and 8 (27%) a moderate-sized infarct (<40%) according to the classification of Pfeffer et al. [6]. Thus, it was logical that median survival time (184 days) was intermediate between the values previously reported for moderate infarcts (230 days) and large infarcts (146 days) [6]. In this context, our data show that 7.5 months after coronary ligation, LVEDP was strongly increased (+392% as compared to S-rats, P<0.01) whereas dP/dt (~27%) and cardiac index (~9%) were decreased. It must be stressed that these figures probably underestimate the cardiac dysfunction in the MI-controls group as a whole, as they were obtained from the animals of that group that survived at 7.5 months, i.e., those in which that function was the less degraded, an assumption that is confirmed by the fact that urinary cyclic GMP in MI-controls at 7.5 months was 60% less than when measured at 1 month. Nevertheless, irbesartan dose-dependently prevented in MI-rats the increase in LVEDP. At the high dose, this effect was significant and the LVEDP value achieved was not significantly different from that measured in S-rats. Irbesartan also increased and almost normalized (as compared to S-rats) dP/dt and cardiac index values. These improvements (vs MI-con-
trols), which occurred despite unchanged MAP values, did not, however, reach statistical significance probably because of (a) the above discussed likely underestimation at 7.5 months of cardiac failure in MI-controls, and (b) the small number of MI-controls. Anyhow, our data clearly indicate that irbesartan improved the status of the cardiac pump function, a finding that was confirmed by the drug-induced dose-dependent and significant reduction (vs MI-controls) in urinary cyclic GMP throughout the study, and that might have been even more spectacular if assessed earlier, i.e., vs MI-controls with a greater degree of CHF. These beneficial hemodynamic effects are thus qualitatively similar to those previously described with ACE inhibitors [6–10] in the same experimental model. This again underlines the major contribution of the renin–angiotensin system interruption to these effects and, although not excluding it, minimizes the possible role of bradykinin in their determinism.

From the morphological point of view, the post-infarction CHF model in rats is characterized by an important cardiac remodeling process including LV dilatation and cardiac hypertrophy and fibrosis development. And indeed, in our MI-controls, LV internal perimeter was increased by 61% after 7.5 months. Cardiac hypertrophy was evidenced by strong increases in HW/BW and RVV/BW. Finally, subendocardial fibrosis developed as shown by the strong increase in subendocardial collagen density (+142%). This profound cardiac remodeling which we observed at animals’ sacrifice and which is present as early as 7 weeks after coronary ligation [30] results in decreased capillary density [31] and hence in a reduced oxygen supply to the cardiac tissue, a potential cause of hypoxia and rhythm disorders [31,32]. This myocardial perfusion deficit, which was clearly evidenced in this study by the strong and significant reduction in LV coronary regional blood flow observed in MI-controls, and the simultaneous impairment of coronary flow reserve that develops in this model [30] clearly contribute to the worsening of LV dysfunction and of heart failure. In this context, irbesartan was found to limit the development of cardiac hypertrophy by 15% for HW/BW and by 20% for RVV/BW (vs MI-controls), an effect that occurred independently of any reduction in afterload as also previously reported with losartan [33]. This result, which confirms earlier data reported with losartan [30], can probably be accounted for by both (a) blockade of cardiac angiotensin II AT₁ receptor-mediated effects on myocyte growth and proliferation, and (b) enhanced stimulation (through the drug-induced increased levels of circulating angiotensin II) of the postulated angiotensin II AT₂ receptor-mediated antiproliferative effects [34]. Irbesartan also increased (as compared to MI-controls) coronary regional blood flows, especially at the low dose, and normalized (as compared to S-rats) coronary regional vascular resistances (which are strongly increased in MI-controls) at both doses. These beneficial effects of irbesartan vs cardiac hypertrophy and on myocardial regional blood flows and resistances are in agreement with those previously reported with losartan and enalapril 7 weeks after coronary ligation in the same experimental model [30] and indicate that AT₁ receptor blockade is, like ACE inhibition, also able to improve myocardial oxygenation. In contrast, irbesartan, whichever the dose, exerted almost no effect vs subendocardial collagen development in our study. In the same experimental model, losartan has been found both to oppose [30,35] and to be almost ineffective [36] vs fibrosis development. The reasons for these discrepancies remain yet unexplained but it must be reminded that in the same context ACE inhibitors administered for 9 to 12 months have systematically been reported to be active, at least vs fibrosis development [8–10,14]. Finally, that irbesartan opposed cardiac hypertrophy but not fibrosis development in our study supports the hypothesis of independent mechanisms of stimulation of both processes [33].

This study was not aimed at investigating the effects of irbesartan on renal hemodynamics and function in CHF. In fact, renal blood flow was not affected in MI-rats, nor were diuresis and creatininuria. In this context, the only detectable effects of irbesartan was a predictable decrease in renal vascular resistance.

In conclusion, chronic angiotensin II AT₁ receptor blockade with irbesartan, started seven days after myocardial infarction, dose-dependently prolongs survival in post-coronary ligation CHF in rats, and this survival prolongation appears to result from the combination of the beneficial effects that the drug exerts on systemic and coronary hemodynamics, on cardiac pump function and vs cardiac hypertrophy development. Chronic AT₁ receptor blockade might thus prove useful and prolong survival in human CHF, a hypothesis that is supported by the preliminary results of the Elite study [20] but which needs further clinical confirmation.

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