

## Advances in the Management of Heart Failure (IV)

## Rationale and Clinical Evidence for the Effects of New Pharmacological Treatments for Heart Failure

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Congestive heart failure is a leading cause of cardiovascular morbidity and mortality, and is the main cause of hospitalization among patients older than 65 years. Moreover, its prevalence is expected to reach nearly epidemic proportions, so that the need for new treatments is clear. In this article we review current and future strategies for the treatment of systolic heart failure that are based on a better understanding of the pathophysiology of this disorder and on the results of controlled clinical trials of different treatments. Drugs under development (phase II and III clinical trials) have been classified into four main groups: neurohumoral antagonists, inotropic agents, modulators of myocardial remodeling, and future approaches to treatment such as gene therapy and anti-apoptotic drugs. We also discuss new strategies for the treatment of diastolic heart failure.

**Key words:** *Heart failure. Neurohumoral antagonists. Inotropic drugs.*

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**Bases y evidencias clínicas de los efectos de los nuevos tratamientos farmacológicos en la insuficiencia cardíaca**

La insuficiencia cardíaca congestiva es una de las principales causas de morbimortalidad cardiovascular y la primera causa de hospitalizaciones en pacientes de más de 65 años. Más aún, dado que se espera que su prevalencia aumente hasta alcanzar proporciones epidémicas, es evidente que necesitamos disponer de nuevas estrategias terapéuticas. En este artículo, revisaremos las estrategias actuales y futuras en el tratamiento de la insuficiencia cardíaca sistólica, que tienen su base en un mejor conocimiento de la fisiopatología de la insuficiencia cardíaca y de los resultados obtenidos en ensayos clínicos controlados con diversas estrategias terapéuticas. Los fármacos actualmente en desarrollo (en fases II y III) los hemos agrupado en 4 grupos: antagonistas neurohumorales, inotrópicos positivos, moduladores del remodelado cardíaco y los futuros tratamientos, entre los que incluimos las terapias génica y antiapoptótica. Por último, analizamos las nuevas estrategias en el tratamiento de la insuficiencia cardíaca diastólica.

**Palabras clave:** *Insuficiencia cardíaca. Antagonistas neurohumorales. Fármacos inotrópicos.*

## INTRODUCTION

Heart failure (HF) is the leading cause of hospitalization in patients over 65 years old and represents

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almost 2% of health costs per year in Spain.<sup>1</sup> Its prevalence is increasing, due in part to increased life expectancy in the general population. It has been calculated that 50 million out of the 1000 million people who live in the 47 countries belonging to the European Society of Cardiology present problems related to HF.<sup>2</sup> Even though in well-designed clinical trials angiotensin-converting enzyme inhibitors (ACEI), beta-blockers and spironolactone have reduced mortality in patients with HF by more than 35%, this continues to be very high. In a study conducted with 66 547 patients hospitalized for HF between

## ABBREVIATIONS

A-II: angiotensin II.  
 ANP/BNP/CNP: natriuretic peptides type A, B, and C.  
 AT1/AT2: angiotensin II receptors.  
 $\beta$ ARK:  $\beta$ 1-adrenergic receptor kinase.  
 [Ca]<sub>i</sub>: intracellular calcium concentration.  
 CTGF: connective growth factor.  
 ACE: angiotensin-converting enzyme.  
 ECE: endothelin-converting enzyme.  
 EGF: epidermic growth factor.  
 NEP: neutral endopeptidase.  
 ET-1: endothelin-1.  
 ET<sub>A</sub>/ET<sub>B</sub>: endothelin receptors type A and B.  
 EF: ejection fraction.  
 GH: growth hormone.  
 HF: heart failure.  
 ACEI: ACE inhibitors.  
 MMP: metalloproteinases.  
 MVO<sub>2</sub>: myocardial oxygen demands.  
 NCX: Na-Ca exchanger.  
 NO: nitric oxide.  
 PAI-1: plasminogen activator inhibitor.  
 PGI<sub>2</sub>: prostaglandin I<sub>2</sub>.  
 PLB: phospholamban.  
 R $\beta$ 1/R $\beta$ 2:  $\beta$ 1- and  $\beta$ 2-adrenergic receptors.  
 SERCA2a: sarcoplasmic reticulum Ca-ATPase.  
 TIMP: metalloproteinase inhibitors.  
 TNF- $\alpha$ : tumor necrosis factor- $\alpha$ .  
 tPA: tissue plasminogen activator.  
 V1/V2: arginin vasopressin receptors.

1986-1995 in Scotland, mortality was 76.5% and 87.6% after 5 years and 10 years, respectively.<sup>3</sup> When this is compared to mortality associated with various cancers, 5 year mortality from HF is higher than colon or prostate cancer in men, and ovarian cancer in women.<sup>4</sup> These results confirm the need for new drugs which will delay the evolution of this disease. The rational development of new drugs should be the logical result of sound knowledge regarding the pathophysiology of the process to be treated. Ignorance in this regard limits the rational design of such strategies and explains the poor outcomes of drugs that had previously raised such high expectations. Thus, between 1950 and 1970 it was thought that HF was a congestive syndrome and was treated with digitalis and diuretics, whereas between 1970 and 1990 it was considered a hemodynamic problem

and thus vasodilator drugs were introduced. In the nineties, the significance of neurohormonal activation in the evolution of HF was recognized, which led to the introduction of  $\beta$ -adrenergic blocking agents and renin-angiotensin-aldosterone system blockers. At present, we know that HF involves changes in cardiac structure and metabolism, as well as in calcium kinetics, and these have become new targets with potential therapeutic interest. The drugs developed more recently belong to four categories (Table 1) which are the object of this review. It should be pointed out that pharmacology is not the only research field in the treatment of HF. New mechanical devices, implantable defibrillators, gene therapy and cell and vessel replacement, as well as teamwork among different medical professionals, are research and development fields as important as pharmacology.

## NEUROHORMONAL MODULATORS

Heart failure is accompanied by marked neurohormonal activation. For example, an increase in the plasma values of mediators (catecholamines, renin-angiotensin-aldosterone system, endothelin 1, vasopressin) involved in vasoconstriction, salt and water retention/edemas, and mitogenic effects has been demonstrated. Similarly, a reduction has been observed in mediators with vasodilator, natriuretic and antiproliferative properties (natriuretic peptides, dopamine, nitric oxide [NO]). In an attempt to counteract neurohormonal activation, the following are being developed.

### Atrial Natriuretic Peptides

These play an important role in the regulation of blood pressure and extracellular volume.<sup>5-7</sup> Three peptides have been described that are synthesized and released in response to atrial distension (type A natriuretic peptide [ANP], 28 amino acids), to increases in ventricular pressure and volume (type B natriuretic peptide [BNP], 32 amino acids) and to endothelial shearing stress (type C natriuretic peptide [CNP], 22 amino acids). More recently, a further 2 peptides have been described: urodilatin, a renal form of ANP, and dendroapsis (DNP), a 38-amino-acid peptide isolated from the green mamba (*Dendroapsis angusticeps*), which is released in the atrium in response to as yet poorly understood stimuli.<sup>7</sup> These peptides act on three types of specific receptors known as A, B, and C. Interaction with C receptors involves the internalization of the peptide and its degradation in cytoplasm. On the other hand, interaction with A and B receptors stimulates particulate guanylate cyclase activity and increases the cellular values of cyclic guanosine monophosphate (cGMP) (Figure 1). As a consequence, they produce

**TABLE 1. New Drugs for the Treatment of Heart Failure\***

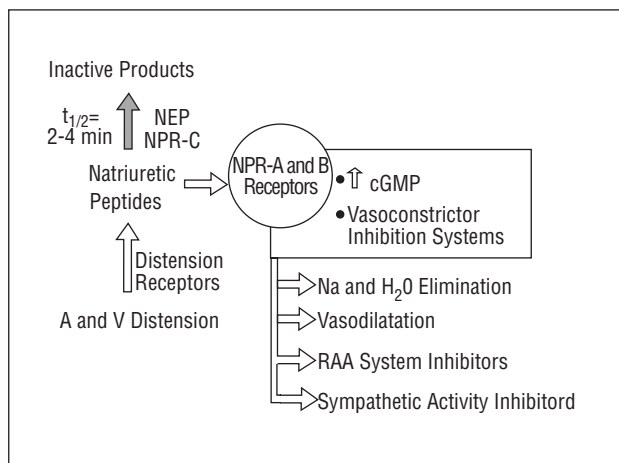
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- A. Neurohormonal modulators
1. Atrial natriuretic peptides: nesiritide, vasonatride, anaritide, mini-ANP
  2. Vasopeptidase inhibitors: mixed inhibitors
    - ACE and NEP inhibitors: omapatrilat, fasidotril, mixampril, sampatrilat, CGS30440, MDL100,240, Z13752A
    - ECE inhibitors: CGS26303, CGS35066, R00687629, FR901533
    - ACE and ECE inhibitors (CGS342256)
  3. Endothelin receptor agonists
    - ETA: darusentan, sitasentan, LU135252
    - ETB: BQ788
    - ETA/ETB: bosentan, enrasentan, tezosentan
  4. Inhibitors of TNF- $\alpha$  and other cytokines: etanercept, pentoxifylline, FR167653, immunoglobulin, enalapril, candesartan, beta-blockers
  5. Vasopressin receptor antagonists: conivaptan, CL-385004, OPC-31260, tolvaptan (OPC-41061), VPA-985
  6. Aldosterone antagonists: eplerenone
  7. Other drugs:
    - New diuretics: atrial natriuretic peptides, adenosine A1 receptor blocker (BG9719) and vasopressin A1 receptor blocker
    - Carnitine, etoxomir, growth hormone, nepicastat, L-thyroxine
  8. Older drugs:
    - ACEI
    - Beta-blockers
    - Angiotensin II AT1 receptor antagonists
    - Aldosterone antagonists
- B. New positive inotropic drugs: levosimendan
- C. Modulation of ventricular remodeling
1. Collagen synthesis inhibitors:
    - Metalloproteinase inhibitors: hydroxamates (batimastat, ilomastat, marimastat, prinomastat), beta-blockers, glucocorticoids, tetracyclines
    - Inhibitors of prolyl-4-hydroxylase, TGF- $\alpha$ , ET-1, kinase, vasopeptidase
    - ACEI, ARA-II
  2. Plasmin antagonists
  3. TIMP inhibitors, adriamycin (that increase collagen degradation)
- D. Other treatments
1. Gene therapy
    - Overexpression of SERCA2a, NCX,  $\beta$ 2 receptors, and adenylate cyclase
    - Inhibition of phospholamban and  $\beta$ ARK1
  2. Anemia as a therapeutic target
  3. Antiapoptotic therapy
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\*ANP indicates type A atrial natriuretic peptide; ARA-II, angiotensin II receptor antagonists; ACE, angiotensin-converting enzyme; ECE: endothelin-converting enzyme; NEP: neutral endopeptidase; ET: endothelin; MMP: metalloproteinases; TIMP: metalloproteinase inhibitors; TNF: tumor necrosis factor.

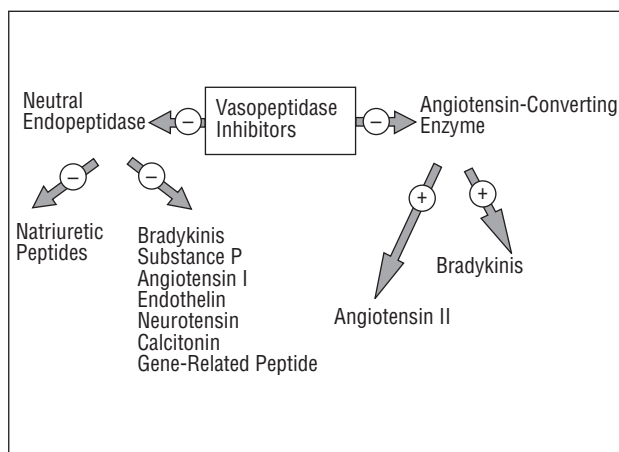
systemic arteriovenous vasodilatation (i.e. decreasing pre- and afterload) and coronary vasodilatation, inhibit sympathetic tone and the renin-angiotensin-aldosterone system (i.e. they diminish the release of renin by the juxtaglomerular cells and of aldosterone by the glomerular area of the suprarenal cortex) and exert antiproliferative action on cardiac muscle cells and vascular smooth muscle cells. In the kidneys, natriuretic peptides produce an increase in renal blood flow, diuresis and natriuresis. Type A natriuretic peptide produces vasodilatation of the afferent glomerular arteriole, vasoconstriction of the efferent glomerular arteriole and increases the speed of glomerular filtration and the fraction of filtration even in patients with acute renal insufficiency and oliguresis.

In the tubules, type A natriuretic peptide inhibits the reabsorption of Na and water produced by angiotensin II (A-II) in the proximal tubule and by vasopressin in the collector tubule. Type C natriuretic peptide has minimal natriuretic effects and venous vasodilator effects, but is a powerful arterial vasodilator and produces mitogenic effects. Dendroapsis produces diuresis and natriuresis.

Atrial natriuretic peptides have a short half-life, since they are rapidly degraded by neutral endopeptidase (NEP) and C receptors. The values of NEP and C receptor density increase in HF patients, whereas those of A and B receptors diminish (Figure 1).



**Fig. 1.** Mechanism of action of atrial natriuretic peptides. A indicates atrial; NEP, neutral endopeptidase; NPR, natriuretic peptide receptors; RAA, renin-angiotensin-aldosterone system; t1/2, plasma half-life; V, ventricular.



**Fig. 2.** Mechanism of action of vasopeptidase inhibitors.

### Role of Natriuretic Peptides in Patients With Heart Failure

The plasma values of atrial natriuretic peptides increase in patients with hypertension, HF, acute myocardial infarction, aortic stenosis, terminal renal failure, cirrhosis and ascites.<sup>5-7</sup> In patients with HF, the increase in plasma values of BNP is more useful than that of ANP or catecholamines to predict patient mortality.<sup>8-10</sup> It can serve as a marker of ventricular dysfunction and of the efficacy of medical treatment for patients with HF.<sup>11,12</sup>

Different options are available to increase the activity of natriuretic peptides which may prove useful in HF (Figure 2): *a*) direct administration of natriuretic peptide (nesiritide); *b*) administration of system non-

peptide agonists; *c*) inhibition of NEP, the enzyme that renders natriuretic peptides inactive, and *d*) administration of simultaneous inhibitors of NEP and ACE (omapatrilate), also known as vasopeptidase inhibitors. Research with NEP inhibitors has been stopped due to their side effects. At present, clinical research only continues with nesiritide which has recently been marketed in several countries.

### Nesiritide

This is human type B natriuretic peptide with 32 amino acids obtained through recombinant technology. When administered intravenously, its effects appear within 15 min and persist for 2–3 h. Nesiritide has a distribution volume of 0.19 L/kg and is biotransformed through NEP, with a half-life of 18–23 min. This is much higher than the 4 min of ANP, although its biological effects persist for 1–4 h.<sup>13,14</sup> Furthermore, unlike ANP, the contribution of C type receptors and NEP to the clearance of BNP is minimal in HF patients.<sup>15,16</sup> It is unnecessary to readjust the dose in the elderly or in patients with renal failure. However, response to BNP declines in patients with ascites/cirrhosis who present a lower response to nesiritide, which means that the dose must be increased to achieve the required hemodynamic response.<sup>17</sup>

### Clinical Trials With Nesiritide

In the PRECEDENT study (Prospective Randomized Evaluation of Cardiac Ectopy with DobutaminE or NaTrecor) on dose calculation, placebo was compared to the effects of continuous i.v. nesiritide infusion (0.25–1 µg/kg bolus followed by infusion of 0.015–0.06 µg/kg/min, respectively). Nesiritide caused a rapid and significant improvement in patients with decompensated HF (functional class II–IV, ejection fraction [EF] ≤35%). At 6 h and 24 h of administration, the group treated with nesiritide presented greater reductions in pulmonary capillary pressure [PCP] and peripheral vascular resistance, as well as a greater increase in cardiac index than the group treated with placebo.<sup>18</sup>

In an open study conducted in 127 patients hospitalized for decompensated HF (PCP ≥18 mm Hg, systolic blood pressure >90 mm Hg, and cardiac index <2.7 L/min/m<sup>2</sup>), nesiritide (0.015 bolus or 0.03 µg/kg/min for 6 h) decreased pre-/postload and PCP, increased minute volume, improved ventricular diastolic function indexes and diminished symptomatology (dyspnea, fatigue) significantly more than placebo. However, it did not increase heart rate nor did it produce proarrhythmic effects.<sup>9</sup>

In the VMAC study (Vasodilation in the Management of Acute Congestive Heart Failure) the effects of nesiritide were compared (2 µg/kg follo-

wed by 0.01 µg/kg/min for 3 h, which in some patients could be increased to doses of 1 µg/kg followed by 0.005 µg/kg/min, up to a maximum of 0.03 µg/kg/min every 3 h) with those of nitroglycerin (at the optimal dose) and placebo.<sup>20</sup> The study was conducted in 489 patients >65 years old with decompensated HF (class III–IV, PCP 28 mm Hg, 18% with EF>40%). After 3 h and 24 h, nesiritide was more effective than nitroglycerin ( $P=.03$ ) and placebo in reducing PCP, but there were no differences between them in reducing dyspnea. Nor were any differences observed in mortality at 7 days and 6 months in patients treated with nesiritide or nitroglycerin. The incidence of adverse reactions was smaller in the nesiritide group; headaches were more frequent with nitroglycerin than with nesiritide (20% vs 4%) and, although there were no differences in arterial hypotension symptoms, the duration of this was greater after nesiritide administration (2.2 h vs 0.7 h in the nitroglycerin group). In a comparative study, in contrast to dobutamine, nesiritide did not aggravate pre-existing ventricular tachycardia nor did it increase the frequency of ventricular extrasystole.<sup>21</sup> At present, new studies are being conducted with nesiritide in patients with acute or chronic decompensated HF.

Nesiritide is contraindicated in patients with arterial hypotension (systolic blood pressure <90 mm Hg), cardiogenic shock, obstructive cardiomyopathy, serious aortic stenosis, constrictive pericarditis, valvular stenosis, pericardiac tamponade, or with low ventricular filling pressure, or serious liver or renal failure.

New peptides obtained by recombinant technology are being currently studied: ANP (28 amino acids), mini-ANP (15 amino acids), anaritide (ANP 25 amino acids), and vasonatride (chimera of ANP and CNP). Anaritide produces vasodilatation of the afferent glomerular arteriole and vasoconstriction of the efferent arteriole, increasing the speed of glomerular filtration in patients with acute renal failure and oliguresis.

### Vasopeptidase Inhibitors

These drugs inhibit various metallopeptidases, such as NEP, that catalyze the degradation of vasodilator and antiproliferative peptides (atrial natriuretic peptides, kinins) and the angiotensin-converting enzymes (ACE) and endothelin 1 conversion enzymes (ECE)<sup>22,23</sup> (Figure 2). The most representative drug is omapatrilat, a mixed ACE/NEP inhibitor.<sup>3</sup> This inhibition increases vasodilator mediators (natriuretic peptides, adrenomedullin, kinins, prostacycline-PGI<sub>2</sub>, NO) and reduces vasoconstrictor mediators (A-II, sympathetic tone). Omapatrilat achieves higher reductions in systolic and diastolic blood pressures than other antihypertensives (amlodipine, lisinopril), regardless of patient age, sex and race. It is absorbed well orally and reaches maximum plasma

concentrations after 0.5–2 h. It has a half-life of 14–19 h, and so can be administered once a day. It is biotransformed into several inactive metabolites that are eliminated through the kidneys and the half-life of the drug is not altered in patients with renal failure. The IMPRESS study (Intramural low Molecular weight heparin for Prevention of Restenosis Study) demonstrated that, in HF patients (functional class II–III), omapatrilat was more effective than lisinopril in improving symptomatology as well as in reducing mortality and rehospitalization due to HF in the medium-term.<sup>24</sup> However, the OVERTURE study (Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events)—carried out in 5770 HF patients in functional class II–IV, with an EF≤30%, a third of whom were diabetic—showed that omapatrilat is not better than enalapril in reducing morbidity and mortality in this subgroup of patients.<sup>25</sup> On the other hand, both this study and the OCTAVE study (Omapatrilat Cardiovascular Treatment Assessment Versus Enalapril), carried out in 25 267 hypertensive patients, confirmed the appearance of angioedema in patients treated with omapatrilat, which stopped the product being marketed.<sup>26</sup> In the OCTAVE study, the incidence of angioedema was three times higher than in patients treated with an ACEI (2.17 vs 0.68%), while in the OVERTURE study it was 0.8% in patients treated with omapatrilat and 0.5% in those treated with enalapril.

### Endothelin 1 (ET-1) Receptor Agonists

Endothelin 1 (ET-1) is the most powerful endogenous vasoconstrictor. It is released by endothelial cells and acts on underlying vascular muscle cells. Endothelin-1 acts on two receptor subtypes, ET<sub>A</sub>, located in vascular smooth muscle cells, myocardium, fibroblasts, kidney and blood platelets, and ET<sub>B</sub>, located in endothelial and vascular smooth muscle cells and macrophages. The stimulation of ET<sub>A</sub> receptors causes vasoconstriction, salt and water retention, cardiac hypertrophy and proliferative effects, and releases norepinephrine and A-II. Stimulation of ET<sub>B</sub> causes vasodilatation by releasing NO and eicosanoids from the endothelial cells, and vasoconstriction by stimulating the vascular smooth muscular cell receptors.<sup>27</sup> Endothelin 1, through stimulation of ET<sub>A</sub> receptors, also stimulates the release of cytokines and growth factors (vascular endothelial growth factor, fibroblastic growth factor, platelet-derived growth factor, TGF-β) and facilitates platelet aggregation. The plasma values of ET-1 are increased in HF, especially in patients with lung congestion, and the expression of ET<sub>B</sub> receptors in the endothelial cells is reduced (ET<sub>A</sub> expression increases or is unchanged). These changes are associated with worse prognosis.<sup>28</sup> In recent years, the effects of selective ET<sub>A</sub> and ET<sub>B</sub> receptor blockers

have been investigated.<sup>29</sup> All these drugs produce beneficial hemodynamic effects during short-term treatment, which raised expectations concerning their usefulness in HF treatment. However, chronic treatment has led to contradictory results.

Of the numerous ET-1 antagonists available, bosentan (Research on Endothelin Antagonists in Chronic Heart Failure [REACH] and Endothelin Receptor Antagonist Bosentan in Lowering

Events in Heart Failure [ENABLE 1 and 2] studies)<sup>30,31</sup> and tezosentan (Randomized Intravenous Tezosentan Study [RITZ 1-4])<sup>32</sup> have not proven better than conventional treatment (diuretics, digoxin, and ACEI) in reducing morbidity and mortality. The REACH-1 study had to be stopped when an increase in transaminase values was detected. In addition, in patients with systolic HF (functional class II–III), treatment with enrasentan for 6 months increased the incidence of adverse reactions versus the placebo group (21% vs 8%), tripled hospitalizations, and tended to increase mortality. Furthermore, the RITZ-4 study reported that tezosentan had a proischemic action in patients with decompensated HF and acute coronary syndromes,<sup>33</sup> whereas the HEAT study (Heart Failure ET<sub>A</sub> Receptor Blockade Trial) showed that darusentan, a selective ET<sub>A</sub> receptor blocker, does not modify hemodynamic parameters in HF patients. Thus, it seems that blocking the ET-1 receptors does not offer many prospects in the future treatment of HF.

## Cytokine Inhibitors

These form a family of proteins that regulate cellular activation, differentiation, growth and death. It includes interleukins (IL), interferons, colony stimulation factors (CSF), chemokines (RANTES, monocyte chemotactic protein [MCP-1]), and cytotoxins (tumor necrosis factor-alpha [TNF- $\alpha$ ]). Cytokines are produced by endothelial cells, T lymphocytes, monocytes and macrophages in response to various stimuli, and participate in the inflammatory process of atheroma plaque and in HF progression.<sup>34</sup> In HF patients the plasma values of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8, MCP-1, and various adhesion molecules increase, regardless of the pathogenesis of the process.<sup>35</sup> The origin of these cytokines is unknown, although it has been suggested that immunological activation induced by endotoxins in the digestive mucous membrane, hemodynamic overload, production of free radicals, and tissue hypoperfusion could be involved.

Tumor necrosis factor-alpha is a proinflammatory protein that seems involved in the genesis of various heart diseases (HF, myocardial infarction [MI], dilated cardiomyopathy, myocarditis). Tumor necrosis factor-alpha depresses cardiac contractility, increases protein catabolism, and causes endothelial dysfunction, in-

flammation, dilatation, fibrosis and cardiac hypertrophy, neurohormonal activation and cardiomyocyte apoptosis.<sup>36,37</sup> Furthermore, it uncouples the  $\beta$ -adrenergic receptors and depresses cardiac contractility, by increasing NO expression and by altering the intracellular kinetics of calcium.<sup>37</sup> In patients with serious HF the plasma values of TNF- $\alpha$  and TNF- $\alpha$  soluble receptors (sTNF-R1 and sTNF-R2) increase, particularly in patients in the decompensation phase or with cachexia. Transgenic mice with TNF- $\alpha$  overexpression have a dilated cardiomyopathy phenotype, HF and lower survival.<sup>38,39</sup>

Etanercept is a fusion protein formed by two soluble ligand molecules for the TNF- $\alpha$ -R2 receptors fused to the Fc fragment of human IgG, that binds to TNF- $\alpha$  and inhibits its binding to its membrane receptors. In short-term studies, it improves symptoms and increases exercise tolerance, but these effects disappear in chronic treatment. This is why the RENAISSANCE (Randomized Etanercept North American Strategy to Study ANtagonism of CytokinEs) and RECOVER (Research into Etanercept: CytOkine Antagonism in VentriculaR dysfunction) studies have been stopped. Recently, the results of the ATTACH (Anti-TNF Therapy Against Congestive Heart failure) study were published, demonstrating that treatment with infliximab (5 and 10 mg/kg for 28 weeks) increased hospitalization and HF mortality at the highest dose tested.<sup>40</sup>

Unlike etanercept, which neutralizes the effects of TNF- $\alpha$ , pentoxifylline inhibits various cytokines (TNF- $\alpha$ , IL-1 $\beta$ , and interferon- $\gamma$ ). In patients with idiopathic dilated cardiomyopathy (functional class II–III; EF <40%) treated with digoxin, ACEI, and carvedilol, pentoxifylline improved symptomatology, EF, and exercise tolerance at a dose which did not modify blood pressure or heart rate (400 mg/12 h).<sup>41</sup> It also lowers Fas/APO-1 values, a surface receptor involved in cardiac apoptosis processes. In patients treated with digoxin and ACEI similar results were observed.<sup>42</sup> However, we do not know its effects in the long-term or on mortality. Other cytokine inhibitors are shown in Table 1. Intravenous immunoglobulin increases EF and exercise tolerance and reduces PCP, IL-1 values and soluble TNF- $\alpha$  receptor density in HF patients.<sup>43,44</sup> These effects have been attributed to the neutralization of microbial antigens, blockage of the Fc receptor, and apoptosis inhibition. Recently, the immunoabsorption technique has been applied to patients with dilated cardiomyopathy, and improvements in EF, functional capacity and ventricular structure have been reported.<sup>45</sup>

It should be noted that other drugs that do not modify (amlodipine, PRAISE [Prospective Randomized Amlodipine Survival Evaluation] study) or reduce morbidity and mortality in HF patients (enalapril, candesartan, or the beta-blockers metoprolol and carvedilol) also inhibit TNF- $\alpha$  values.

### Vasopressin Receptor Antagonists

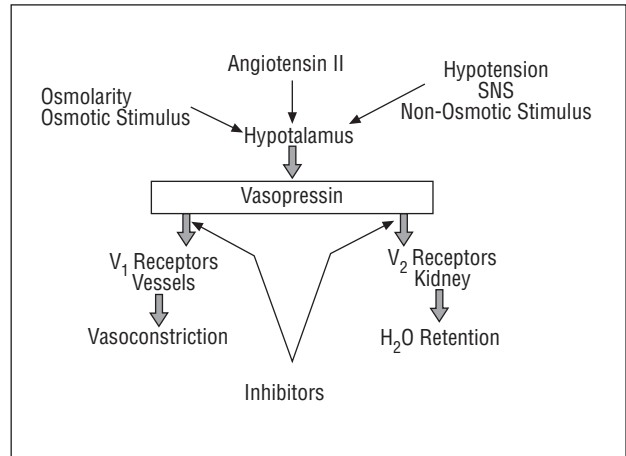
This neurohormone is synthesized in the hypothalamus and is stored and released by the neurohypophysis in response to different stimuli (increase in plasma osmolarity, hypotension, increase in A-II values).<sup>46</sup> Vasopressin acts on two types of receptors: V<sub>1</sub> (subtypes V<sub>1A</sub> and V<sub>1B</sub>) and V<sub>2</sub> (Figure 3). The V<sub>1A</sub> receptors are located in the vascular smooth muscle cells, blood platelets, mesangial cells, collector tubule, central nervous system, and liver. Its stimulation causes arteriovenous vasoconstriction and cardiac hypertrophy. The stimulation of V<sub>1B</sub> receptors, located in the anterior hypophysis, facilitates the release of ACTH, whereas stimulation of V<sub>2</sub> receptors, located in the collector tubule, cause greater reabsorption of free water, water retention and hyponatremia by dilution.

Conivaptan (YM-087) is a V<sub>1A</sub> and V<sub>2</sub> receptor antagonist that increases diuresis and clearance of free water with minimum Na loss, thereby diminishing urinary osmolarity.<sup>47</sup> Thus, it would be useful in patients with HF, edemas and hyponatremia who frequently present resistance to thiazidic or loop diuretics. In fact, conivaptan increases urinary excretion even in patients resistant to furosemide. However, its effects on neurohormonal activation or morbidity and mortality in HF patients remain unknown. During treatment dizziness, hypotension, polyuria, constipation and thirst appear. Two selective V<sub>2</sub> receptor antagonists (OPC-41061 or tolvaptan and VPA-985) are currently under clinical development in HF patients. However, the effects of all these drugs on mortality in such patients remains unknown.<sup>46</sup>

### Aldosterone Antagonists

Ventricular pressure overload increases extracellular matrix production by cardiac fibroblasts and reduces ventricular elastance in patients with hypertension, cardiac hypertrophy, and HF. Angiotensin-II (via AT1 receptors) and aldosterone increase interstitial and cardiac perivascular fibrosis, although the release of aldosterone is independent of A-II release and increases the extracellular matrix, even in the presence of an ACEI. Furthermore, aldosterone causes renal retention of Na and greater excretion of K and Mg, baroreceptor dysfunction, strengthens catecholamine effect and increases ventricular arrhythmogenicity.<sup>48,49</sup>

Spironolactone is an aldosterone receptor antagonist. In the RALES study (Randomized Aldactone Evaluation Study) the effects of suppressive doses of spironolactone (25 mg/day) were analyzed in patients with serious HF (functional class III–IV) treated for 24 months with triple therapy. Functional class improved, hospitalizations were reduced and survival increased



**Fig. 3.** Mechanisms that regulate vasopressin synthesis and effects mediated by vasopressin when interacting with its specific receptors.

by 30%.<sup>50</sup> Gynecomastia, hyperkalemia, and increases in plasma creatinine values can appear during treatment and should be suspended if hyperkalemia is >6 mmol/L and creatinine values >4 mg/dl. These results indicate that spironolactone should form part of the treatment for serious HF (functional class IV). The finding that spironolactone lowers the plasma values of procollagen type III aminoterminal peptide confirms the importance of fibrosis inhibition in its effects.<sup>51</sup>

Recently, the EPHEBUS study (Eplerenone Neurohormonal Efficacy and Survival Study) demonstrated that eplerenone, another aldosterone antagonist, reduced total mortality, cardiovascular mortality and sudden death, as well as hospitalizations due to HF in patients with previous MI and HF.<sup>52</sup> Eplerenone has high affinity for mineral corticoid receptors and low affinity for estrogen and progesterone receptors (<1% and 0.1% of that presented by spironolactone), which entails a lower incidence of gynecomastia (0.5%), impotence (0.1%) and hyperkalemia (3.4%) than with spironolactone. An additional advantage of both aldosterone antagonists is that their beneficial effects persist in patients treated with beta-blockers and ACEI.

### Other Drugs

Currently, the safety and efficacy of the following drugs are being evaluated:

#### Adenosine A1 Receptor Antagonists

The stimulation of adenosine A1 receptors, located in the afferent arteriole, causes vasoconstriction and reductions in renal blood flow, and increases the reabsorption of Na in the proximal and distal tubules. Furthermore, the increase in Na load in the distal tu-

bule causes an increase in adenosine values that reduces the speed of glomerular filtration via a tubuloglomerular feedback process. The A1 receptor antagonists cause selective vasodilatation of the afferent glomerular arteriole and natriuretic action as a consequence of their effects on the proximal and distal tubules. BG9719/CVT-124 is an A1 receptor antagonist that has been demonstrated to cause diuresis and maintain glomerular function in clinical trials.<sup>53-55</sup> In HF patients treated with an ACEI, BG9719 increases the volume and excretion of Na in urine. The administration of BG9719 with furosemide also increases diuresis and glomerular filtration speed. Furthermore, this combination prevents the reduction in creatinine clearance produced by furosemide, which confirms that BG9719 can prevent deterioration in renal function in HF patients and the reduction in glomerular filtration produced by loop diuretics.<sup>55</sup>

### *New Sympathetic Drugs*

Nepicastat is a dopamine  $\beta$ -hydroxylase inhibitor that reduces norepinephrine synthesis and the stimulation of adrenergic receptors  $\alpha_1$ ,  $\beta_1$ , and  $\beta_2$ . Furthermore, it increases dopamine release from the sympathetic nerve terminals which causes renal vasodilatation. In dogs with chronic HF (EF, 30%-40%), nepicastat at low doses normalizes plasma concentrations of norepinephrine, attenuates ventricular remodeling and prevents systolic dysfunction progression. However, at high doses, that normalize plasma norepinephrine values, there is no significant improvement in ventricular function and morphology.<sup>56</sup> This finding suggests that a certain degree of sympathetic activation is necessary even in the presence of HF. The combination of low doses of nepicastat and enalapril increased minute volume and prevented ventricular remodeling, producing a beneficial effect similar to that observed when combining beta-blockers and ACEI.

Nolomirole (CHF-1024) is a  $\alpha_2$  and DA2 presynaptic receptor agonist that reduces the release of norepinephrine and the sympathetic tone.<sup>57</sup> The ECHOS study (EchoCardiography and Heart Outcome Study) analyzed the effects of nolomirole on a population of patients similar to the study DIAMOND (Danish Investigations of Arrhythmia and Mortality On Dofetilide).

### *Drugs That Improve Cardiac Metabolism*

Fatty acids are the main source of energy during cardiac ischemia, but they uncouple oxidative phosphorylation and increase myocardial demands for  $O_2$ . In these circumstances, partial fatty acid oxidation (pFOX) inhibitors increase glucose oxidation and could increase cardiac efficiency. Increases in glucose

oxidation can be obtained with several enzyme inhibitors: etomoxir (carnitine palmitoyl transferase 1), oxfenicine, methyl palmoxyrate, S-15176, perhexiline, aminocarnitine (carnitine palmitoyl transferase 1), hydrazonopropionic acid (carnitine acylcarnitine translocase), MET-88 ( $\gamma$ -butyrobetaine hydroxylase), trimetazidine and ranolazine (acetyl-CoA C-acyltransferase), hypoglycin (butyryl coenzyme A dehydrogenase), and dichloroacetate (pyruvate dehydrogenase kinase).

Etomoxir is an inhibitor of the carnitine palmitoyl transferase 1 (CPT-1), a mitochondrial enzyme that inhibits the transport of long-chain acyl coenzyme A compounds, while promoting glucose transport toward the mitochondria. In animal models, etomoxir reverses the changes in fetal gene expression, preserves cardiac function and prevents ventricular dilatation.<sup>58</sup> In HF patients (functional class II-III), the administration of etomoxir for 3 months improves EF (from 21.5% to 27%) and cardiac minute volume, and diminishes PCP; that is, ventricular systolic function improves.<sup>59,60</sup> Given that the drug does not modify blood pressure (which suggests that it does not alter afterload), the authors think that the increase in ventricular function produced by etomoxir might be due to an increase in the expression of SERCA2a. However, long-term controlled studies are needed to reliably confirm the effects of etomoxir.

Ranolazine is a pFOX inhibitor that suppresses the oxidation of fatty acids and improves ventricular function in animal models. This improvement can be attributed to better ATP synthesis by consumed  $O_2$  and/or to a more efficient use of ATP by cardiac cells.<sup>61,62</sup>

Growth hormone (GH) causes hemodynamic and functional improvement in HF patients secondary to dilated cardiomyopathy although its long-term effects are unknown. In patients with type I diabetes, nephropathies or cachexia it reduces GH receptor expression and resistance to GH might appear.<sup>63</sup>

### **Older Drugs**

Angiotensin-converting enzyme inhibitors, beta-blockers, angiotensin II AT1 receptor antagonists (ARA-II), and spironolactone have shown their safety and efficacy in patients with HF. It has been demonstrated that ACEI reduce mortality and morbidity in patients with ventricular systolic dysfunction, with or without HF symptoms, due to heart attack or chronic evolution (SAVE, TRACE, AIRE, CONSENSUS, VHEFT, SOLVD). In addition, they prevent or delay the appearance of symptoms in patients with asymptomatic HF.<sup>64</sup> These studies also show that ACEI reduce the risk of myocardial infarction and the incidence of atrial fibrillation.<sup>65</sup> The protective anti-ischemic action of ACEI has been confirmed in the HOPE study<sup>66</sup> and their vasculoprotective action in the PEACE and EUROPE studies.



Three beta-blockers—carvedilol (COPERNICUS, CAPRICORN), metoprolol (MERIT-HF), and bisoprolol (CIBIS II)—have demonstrated reductions in morbidity and mortality in HF patients in various degrees of functional deterioration.<sup>64,67,68</sup> Recently, the COMET study (Carvedilol Or Metoprolol European Trial) reported greater reductions in mortality in HF patients with carvedilol, thus making it preferable to metoprolol. However, the relative efficacy of bisoprolol and carvedilol is unknown.<sup>69</sup> This study has shown that carvedilol prolongs the life of HF patients by 1.4 years more than  $\beta_1$ -selective blocking agents. Currently, the SENIORS study (Outcomes and Rehospitalisation in Seniors with Heart Failure) is investigating the effects of nebivolol on 2000 patients  $\geq 70$  years old with an EF  $\leq 35\%$ , and the BETACAR study (BETAxolol vs CARvedilol in chronic heart failure) is investigating the effects of carvedilol and beta-xolol. The CIBIS 3 study is comparing the possible impact of initiating treatment with bisoprolol or ACEI. A large percentage of HF patients present areas of hibernating myocardium and according to the CHRISTMAS study, carvedilol has a beneficial effect on these patients.<sup>70</sup>

However, despite their undoubted efficacy, ACEI and beta-blockers are prescribed infrequently (in less than 60% of patients) and at doses lower than those used in clinical trials. On the other hand, their usefulness in patients with diastolic dysfunction and in black people remains unclear. At present, in the A-HeFT (African American Heart Failure) study the effects of the hydralazine-isosorbide dinitrate combination on black patients with HF (functional class III–IV) are being compared to placebo.

The safety and efficacy of ARA-II have been compared to those of ACEI and placebo (Table 2). In the ELITE II (Evaluation of Losartan in the Elderly), OPTIMAAL (Optimal Trial in Myocardial infarction with the Angiotensin II Antagonist Losartan), ValHeFT (Valsartan in Heart Failure Trial), and VALIANT (Valsartan In Acute Myocardial Infarction Trial) studies no differences were observed in mortality in patients in functional class II–IV treated with captopril and losartan or valsartan, respectively.<sup>71–75</sup> However, in the ValHeft study a reduction in rehospitalizations was reported when comparing valsartan with placebo. Recently, the CHARM study (Candesartan cilexetil in Heart failure Reduction in Mortality and morbidity) investigated the effects of candesartan in three substudies: 2300 HF patients with EF  $< 40\%$  treated with ACEI, 1700 patients with EF  $< 40\%$  and intolerance to ACEI, and 2500 patients with EF  $> 40\%$  not treated with ACEI.<sup>76–79</sup> The primary aim was to study mortality from any cause, cardiovascular mortality and hospitalizations due to HF. The results of the study showed that: *a*) in patients with symptomatic HF, EF  $< 40\%$ , and intolerance to

ACEI, candesartan reduces mortality and cardiovascular morbidity (CHARM-alternative). In this subgroup, the leading causes of intolerance to ACEI were cough (72%), symptomatic arterial hypotension (13%), or renal dysfunction (12%); *b*) the addition of candesartan to an ACEI (and a beta-blocker) causes a reduction in cardiovascular morbidity and mortality in HF patients, although not in global mortality (CHARM-added). This benefit is obtained with relatively few adverse effects, although there is an increase in the risk of hypotension, hyperkalemia, and renal dysfunction, and *c*) in HF patients in functional class II–IV with EF  $> 40\%$  treated with an ACEI, candesartan (CHARM-preserved) does not modify cardiovascular mortality, but does reduce hospitalizations for HF and the appearance of diabetes mellitus.

## NEW POSITIVE INOTROPIC DRUGS

Positive inotropic drugs have been widely utilized in the treatment of HF. However, the majority (sympathomimetic: dopamine, dobutamine, floxequinan; phosphodiesterase III inhibitors: amrinone, milrinone, enoximone, vesnarinone) increase mortality. The exception is digoxin which, in the DIG study, did not modify mortality, but did reduce symptomatology and hospitalization for HF.<sup>80</sup> This increase in mortality has been attributed to their ability to increase intracellular values of cAMP, either by increasing adenylate cyclase ( $\beta$ -adrenergic agonists) activity or by inhibiting its degradation (phosphodiesterase III inhibitors). The increase in cAMP cardiac values activates protein kinase A that phosphorylates L-type Ca channels and increases the influx of Ca through them and the intracellular concentration of free Ca ( $[Ca]_i$ ). The increase of  $[Ca]_i$  in contractile proteins increases contractility, but also heart rate, myocardial  $O_2$  (MVO<sub>2</sub>) demand, and necrosis and cardiac apoptosis processes. All these effects increase the incidence of ischemic heart disease, high-risk ventricular arrhythmias and patient mortality. Despite these drawbacks, there is renewed interest in the use of low-dose phosphodiesterase III inhibitors in combination with beta-blockers in advanced HF patients.<sup>81</sup> These patients depend on sympathetic tone to maintain cardiac function and therefore they tolerate beta-blockers poorly; in these circumstances phosphodiesterase III inhibitors facilitate treatment with beta-blockers which counteract their proarrhythmic effects. Whether this drug combination is of real use remains to be demonstrated.

## Drugs That Increase Sensitivity of Contractile Protein to Calcium

Troponin (Tn) is a globular protein which exists as 3 subunits: T (38 kDa), C (18 kDa), and I (22 kDa).

**TABLE 2. Studies of Morbidity and Mortality With Angiotensin II Receptor Antagonists in Heart Failure\***

Study <sup>a</sup>	Angiotensin II Receptor Antagonist	Heart Failure	Global Mortality	Rehospitalization
ValHeft	Valsartan/placebo	CHF	NS	V better than <i>P</i>
CHARM Alternative <sup>c</sup>	Candesartan/placebo	CHF	C better than <i>P</i> <sup>b</sup>	C better than <i>P</i>
CHARM Added	Candesartan/placebo	CHF	C better than <i>P</i> <sup>b</sup>	C better than <i>P</i>
CHARM Preserved	Candesartan/placebo	Various	NS	C better than <i>P</i>
ELITE II	Losartan/captopril	CHF	NS	NS
OPTIMAAL	Losartan/captopril	VD/HF post-AMI	NS	NS
VALIANT	Valsartan/captopril	VD/HF post-AMI	NS	NS
VALIANT	Valsartan + captopril/captopril	VD/HF post-AMI	NS	NS

\*ARA-II indicates angiotensin II receptor antagonists; C, candesartan; VD, ventricular dysfunction; AMI, acute myocardial infarction; HF, heart failure; CHF, congestive heart failure; ACEI, angiotensin II receptor antagonist inhibitors; NS, nonsignificant; *P*, placebo. <sup>a</sup>Patients with systolic dysfunction and reduced ejection fraction, except in CHARM-Preserved. <sup>b</sup>Cardiovascular mortality. <sup>c</sup>Without ACEI in the ARA-II group.

Troponin C lies between TnT and TnI and is the subunit to which Ca binds. During diastole ( $[Ca]_i=0.1 \mu\text{mol/L}$ ), actin is covered by TnI and tropomyosin, which forms a complex that prevents actin binding to the globular heads of the myosin and increases its ATPase activity. During systole  $[Ca]_i$  (0.6–1  $\mu\text{mol/L}$ ) increases and Ca interacts with TnC producing a conformational change, such that the TnI rotates and dissociates from the actin and tropomyosin. This makes it possible to free active zones in the actin surface which become the points where cross-linking with the myosin head takes place.<sup>82</sup> The formation of cross-links between the actin and myosin allows the thin actin filaments to slide over the thick ones of myosin, shortening the length of the sarcomere during systole. During relaxation  $[Ca]_i$  decreases, and this facilitates TnI and tropomyosin returning to their initial position and prevents actin-myosin coupling and the formation of cross-links.

**Levosimendan**

A new group of positive inotropic drugs has appeared recently that bind to TnC and increases its sensitivity to calcium. Levosimendan binds to the N-terminal domain of TnC, which is the point at which Ca binds to produce the contractile response, and prolongs the conformational changes produced in TnC by increasing  $[Ca]_i$ .<sup>83,84</sup> As a consequence, it accelerates the formation and number of cross-links between the actin and myosin. The result is that, in the presence of levosimendan, the contractile force developed increases for any concentration of intracellular Ca, and this inotropic effect is not accompanied by changes in cardiac relaxation.<sup>84,85</sup> The effects of levosimendan do not modify  $[Ca]_i$ , consumption of ATP, myocardial demands for O<sub>2</sub> or ventricular relaxation. Thus, it has been suggested that the persistence of cross-linking would be the most efficient way to in-

crease contractility in terms of energy.<sup>83</sup> The effect of levosimendan is regulated by  $[Ca]_i$ , such that when decreasing during diastole, the drug dissociates from TnC and therefore does not delay ventricular relaxation.<sup>84</sup>

In vascular smooth muscle cells, levosimendan activates ATP-sensitive K channels.<sup>86,87</sup> Consequently, it hyperpolarizes cellular membrane potential, diminishes the probability of the L-type Ca entry channels opening, and increases the output of Ca via the Na-Ca exchanger. The result of these effects is a reduction of  $[Ca]_i$  in vascular smooth muscular cells that translates into systemic arteriovenous, pulmonary artery, and coronary vasodilatation that reduces pre/afterload.

In animal models and in HF patients, levosimendan produces a dose-dependent increase in cardiac contractility, stroke volume, minute volume and systolic blood pressure of the left ventricle, but does not modify or even accelerate the speed of ventricular relaxation.<sup>84,87</sup> This increase in contractility is maintained even in the presence of dopamine. Furthermore, in contrast to dobutamine, the increase in ventricular contractility produced by levosimendan persists even in patients treated with  $\beta$ -adrenergic blocking agents.<sup>88</sup> In animal models of coronary ischemia-reperfusion, levosimendan does not modify myocardial O<sub>2</sub> consumption, but does increase coronary blood flow, an effect that is counteracted with glibenclamide, a inhibitor of selective K (ATP) channels, which confirms their key role in coronary vasodilatation.<sup>84</sup>

*Patients with stable HF.* When administered i.v. to patients with systolic HF (functional class III–IV), levosimendan improves hemodynamic alterations (increases minute volume and diminishes pulmonary capillary and right atrial pressures), dyspnea and fatigue, and shortens hospital stay and reduces readmissions.<sup>89,90</sup> Levosimendan does not modify catecholamine plasma values, but in patients in functional

class III–IV it lowers pulmonary ET-1 values.<sup>84,91</sup>

*Patients with decompensated HF.* In the RUSSLAN study (Randomized sUdy and Safety and effectiveness of in Levosimendan patients with left ventricular failure after an Acute myocardial iNfarct), the effects of levosimendan (6, 10, 12, or 24 µg/kg bolus for 10 min followed by an infusion of 0.1, 0.2, or 0.4 µg/kg/min for 6 h) were compared with placebo in 502 patients with HF after acute myocardial infarction.<sup>92</sup> The primary aim of the study was to identify the proportion of patients who developed significant arterial hypotension or ischemia. Differences were only found in the incidence of hypotension or ischemia between the group treated with the maximum dose of levosimendan and the placebo group. Levosimendan produced a dose-dependent significant reduction in the combined risk of worsening HF and death compared to the group treated with placebo, at 6 h treatment (2.0% vs 5.9%;  $P=.033$ ) as well as at 24 h (4.0% vs 8.8%;  $P=.044$ ). After 14 days, total mortality was less in the active group than in the placebo group (11.4% vs 19.6%,  $P=.029$ ).

The LIDO study (Levosimendan Infusion versus Dobutamine) compared the administration of levosimendan (24 µg/kg i.v. followed by an infusion of 0.1 µg/kg/min) and dobutamine (5 µg/kg/min) for 24 h in patients with decompensated HF.<sup>88</sup> If the cardiac index increased less than 30% after 2 h of infusion, levosimendan dose was increased up to 0.2 µg/kg/min and dobutamine up to 10 µg/kg/min. Both drugs improved symptomatology, reduced PCP and increased cardiac index. However, more patients in the levosimendan group showed increases in minute volume  $\geq 30\%$  and a reduction in PCP  $\geq 25\%$  than in the dobutamine group (28% vs 15%;  $P=.02$ ). There were also more patients in the group treated with levosimendan (28% vs 15%) who did not need rescue medication after 24 h. Furthermore, levosimendan reduced mortality more at 180 days (27% vs 38%) and significantly increased the number of days out of hospital at 6 months follow-up (133 days vs 157 days;  $P=.027$ ). The total incidence of adverse reactions and of tachyarrhythmias was greater in the group treated with dopamine, although the group treated with levosimendan presented more arterial hypotension episodes. In fact, in the LIDO and RUSSLAN studies, it was not possible to demonstrate that levosimendan increases the incidence of cardiac arrhythmias.

Currently, 2 studies are investigating the effects of levosimendan on HF patients. In the SURVIVE study, 180-day total mortality is being compared to dobutamine, and in the REVIVES study the efficacy and safety of levosimendan is being compared to placebo.

## MODULATION OF VENTRICULAR REMODELING

Ventricular remodeling in patients with arterial hypertension and chronic HF or previous infarction contributes to disease development, with progressive functional and anatomical deterioration and, ultimately, is related to a worse prognosis.<sup>93,94</sup> Remodeling is a complex and dynamic process that develops slowly and progressively, in which mechanical factors intervene (increases in intraventricular pressure and neurohormones and changes in gene expression). The cardiac extracellular matrix includes the collagen fiber network (types I and III), the basal membrane and the proteoglycans. The matrix represents a dynamic balance between mechanisms that facilitate their synthesis and degradation.

The metalloproteinases (MMP) are a large group of enzymes (collagenases [MMP-1, 8, 13, and 18], gelatinases [MMP-2 and 9], stromelysins [MMP-3, 10, and 11], matrilysins [MMP-7], metalloelastase [MMP-12], and membrane-type [MMP-14-17]) released by fibroblasts and cardiac, vascular smooth muscle and endothelial cells. They regulate the composition of the extracellular matrix.<sup>94,95</sup> The expression of MMP is stimulated by free radicals, cytokines (TNF- $\alpha$ , IL-1), growth factors (connective tissue [CTGF], epidermal [EGF], platelet-derived [PDGF] or fibroblastic [FGF]) and A-II (through AT2 receptors). There are also endogenous metalloprotein inhibitors (TIMP) and pharmacological metalloprotein inhibitors (tetracyclines, anthracyclines, phosphonamidates, hydroxamates [batimastat, ilomastat, marimastat, prinomastat], beta-blockers, glucocorticoids, retinoides, and heparin).<sup>94,95</sup> MMP and TIMP coexpress in various types of inflammatory and endothelial cells and fibroblasts. TIMP-1 and 2 are diffusible and are found in the interstitial compartment, whereas TIMP-3 is found bound to different components of the extracellular matrix. Two types of MMP-3 inhibitors have been described that act as zinc-chelating agents in the catalytic domain of the enzyme: galaridin, which binds zinc to a hydroxamic acid group; and PD 180557 and PD 166793, that chelate zinc due to the presence of carboxylic acid groups.<sup>96</sup>

Metalloproteinases and TIMP are closely linked to the remodeling process. During the ventricular remodeling process, which facilitates progression toward HF and cardiac rupture, there are increases in the expression of MMP-1, 2, 3, and 9, and reduced activity of TIMP-1 and 3 in atheroma plaque and after angioplasty. There is also reduced TIMP-1, 3 and 4 expression in patients with ischemic heart disease, and in mice with TIMP-1 deficiency ventricular hypertrophy appears as well as increases in end-diastole volume.<sup>94-101</sup> All this facilitates collagen accumulation and cardiac fibrosis. Furthermore, some poly-

morphisms of MMP-3, 9, and 12 increase the incidence of ischemic heart disease and aortic aneurysms, and deletions of MMP-9 inhibit ventricular dilatation after myocardial infarction.<sup>95</sup> In addition, in animal models, TIMP reduce ventricular dilatation and improve the post-infarction ventricular function.<sup>94,95</sup> In transgenic mice, deletion, or overexpression of MMP regulates the cardiac architecture.<sup>101-103</sup> Taken as a whole, this evidence suggests that the inhibition of cardiac MMP could prevent ventricular dysfunction and delay HF progression. However, the factors that regulate the activity of each MMP in the human myocardium and their role remains unknown, in addition to whether the improvement in ventricular function involves a reduction in patient mortality.<sup>94,95</sup> Despite this, we know that the reduction in ventricular hemodynamic overload reduces MMP and increases TIMP.

In an attempt to reduce cardiac fibrosis and its adverse effects on ventricular function, various drugs have been utilized. These include ACEI, pirfenidone (that inhibits the synthesis of collagen induced by TGF- $\beta$  and various cytokines), pentoxifylline (that increases the cardiac values of adenosine) and ALT-711 (4.5-dimethylthiazolium chloride, that breaks collagen cross-linking). In turn, some drugs and cellular mediators worsen the clinical picture of remodeling, whether by increasing collagen synthesis (GH, bradykinin inhibitors), or by inhibiting its degradation (recombinant TIMP, phenytoin, retinoids, MMP inhibitors). Angiotensin-converting enzyme inhibitors, ARA-II, and spironolactone reduce extracellular matrix collagen, and the  $\beta$ -adrenergic blocking agents reduce MMP.<sup>94,104</sup> Angiotensin II receptor antagonists also lower the values of prolyl-4-hydroxylase (P4H).<sup>102</sup> Nitrates preserve collagen in the infarct area and prevent the reduction of collagen during reperfusion.<sup>94</sup> Endothelins increase collagen synthesis and lower MMP values,<sup>94</sup> while endothelin receptor blocking agents reduce post-infarction scarring of the myocardium. Bradykinin increases MMP and reduces collagen, whereas adenosine, which increases the cardiac values of cAMP, cGMP and NO, reduces fibrosis.<sup>94</sup>

The plasminogen/plasmin system participates in ventricular remodeling, since it activates various MMP and releases TGF- $\beta$ , which inhibits cellular proliferation and collagen clustering.<sup>95</sup> This system can be amplified by urokinase and tissue plasminogen activator (tPA) or inhibited by plasminogen activator inhibitor (PAI-1). In conclusion, the modulation of cardiac remodeling is an interesting therapeutic target, even though current treatment is far from ideal. From a practical standpoint, and up to the present, a reduction in ventricular remodeling has only been demonstrated with ACEI and beta-blockers.

## OTHER TREATMENTS

### Genetic Therapy

Alterations in the intracellular metabolism of Ca and in the intracellular signaling pathway of the  $\beta$ -adrenergic receptors have been identified in HF patients.<sup>105,106</sup> These constitute possible therapeutic targets although there is still no clinical evidence to support their introduction into clinical practice.

### *Alterations in the Cellular Kinetics of Ca*

Calcium ions perform a central role in cardiac contraction-relaxation.<sup>106,107</sup> In HF there are various alterations in the cardiac metabolism of Ca that lead to an increase in  $[Ca]_i$  during diastole, secondary to reduced activity of ATPase dependent on calcium from the sarcoplasmic reticulum (SERCA2a), and to increased activity of the sarcolemmal Na-Ca exchanger (NCX). In animal models of HF, SERCA2a mRNA and its enzymatic activity decrease. Both effects are involved in the transition from compensatory hypertrophy to HF. The activity of SERCA2a is regulated by phospholamban (PLB). When PLB is not phosphorylated, it inhibits SERCA2a activity, whereas the phosphorylation of PLB increases SERCA2a affinity for Ca and Ca uptake by the sarcoplasmic reticulum. Furthermore, in failing myocardium the values of phosphorylated PLB decrease and the expression and activity of a protein phosphatase 1 increase. As a consequence, most PLB is dephosphorylated, which diminishes SERCA2a affinity for Ca, increases diastolic  $[Ca]_i$  and delays relaxation.<sup>108,109</sup> On the other hand, the inactivation of phospholamban or the inhibition of its expression increase cardiac contraction and SERCA2a affinity for Ca, which entails an acceleration in cardiac relaxation.<sup>108,109</sup> SERCA2a activity can be increased by inhibiting PLB expression, increasing the expression of dominant negative mutants of PLB, administering PLB inhibitors, or increasing SERCA2a expression. Gene transfer of SERCA2a increases contractility and accelerates cardiac relaxation,<sup>105,110-112</sup> but it also increases sarcoplasmic reticulum Ca content and prolongs ventricular repolarization which facilitates the appearance of late afterpotentials in failing myocardium. Beta-blockers, etomoxir and MET-88 increase SERCA2 expression, and this effect could be involved in their capacity to improve ventricular function.<sup>113</sup>

Ventricular hypertrophy, dilated cardiomyopathy, aortic stenosis, hypothyroidism or aging diminish SERCA2a activity and increase PLB activity, thereby delaying relaxation, whereas in hyperthyroid patients the opposite occurs. On the other hand, the increase in cAMP values induced after the stimulation of  $\beta_1$ -adrenergic receptors ( $R\beta_1$ ), or the inhibition of cardiac phosphodiesterase III, phosphorylate PLB and reduce

its inhibitory action on SERCA. Furthermore, captopril and  $R\beta_1$  agonists increase SERCA2a activity and/or diminish that of PLB, thereby accelerating ventricular relaxation.

In HF there is increased expression of the *NCX1* gene, which codes the Na-Ca exchanger, and this increases the influx of  $Ca^{2+}$  into the cardiac myocyte, prolongs action potential duration and facilitates the appearance of early and late afterpotentials, i.e. the appearance of cardiac arrhythmias in the failing myocardium.<sup>114</sup>

### Regulation of the $\beta$ -Adrenergic Receptor Signaling Pathways

The  $\beta$ -adrenergic receptor signaling pathway plays an important role in cardiac contractility control, both in the normal and failing myocardium. In HF there are various alterations in this pathway: a reduction in the density of  $\beta_1$ -adrenergic receptors, inhibition of adenylyl-cyclase activity, adrenergic receptor and Gsa protein uncoupling, greater  $\beta_1$ -adrenergic receptor kinase activity ( $\beta$ ARK, that desensitizes the  $\beta_1$ - and  $\beta_2$ -adrenergic receptors) and an increase in Gi protein expression.<sup>115</sup>

Ventricular contractility is increased in mice that overexpress  $\beta_1$ - and  $\beta_2$ -adrenergic receptors, adenylyl cyclase and Gs $\alpha$  protein, or where  $\beta$ ARK or the phospholamban-coding gene is inhibited. However, sustained  $\beta$ -adrenergic stimulation or overexpression of adrenergic  $\beta_1$ -receptors or Gs $\alpha$  proteins produce a cardiomyopathy phenotype characterized by hypertrophy and fibrosis, induce cardiac apoptosis and increase the incidence of arrhythmias;<sup>116,117</sup> furthermore, they induce the expression of proinflammatory cytokines (TNF- $\alpha$ , IL-1, and IL-6), which lowers contractility even more and facilitates cardiac expansion.<sup>118</sup> In turn, overexpression of adrenergic  $\beta_1$ -receptors diminishes contractility and heart rate and produces a cardiomyopathy phenotype.<sup>119,120</sup> It is not, then, surprising that drugs that increase sympathetic tone (dobutamine, prenalteol, xamoterol) facilitate HF progression and shorten the survival of HF patients. On the other hand, adrenergic receptor desensitization in HF patients is associated with an increase in  $\beta$ ARK1 expression. Overexpression of a  $\beta$ ARK1 inhibitor restores coupling among receptors and Gsa protein and increases cardiac contractility, which offers a new therapeutic option.<sup>121</sup> In mice undergoing chronic treatment with carvedilol, cardiac adrenergic  $\beta_1$ -receptor coupling increases, which is associated with a lower expression of  $\beta$ ARK1.

### Anemia as a Therapeutic Target

Anemia is a factor that contributes to HF symptomatology, and in HF patient records reductions in hemoglobin have been related to a worse prognosis. A

high percentage of HF patients are anemic, and treatment with iron supplements and erythropoietin improves symptoms and morbidity.<sup>122</sup> At present, various studies are investigating the effects of this treatment on morbidity and mortality in HF patients.

### Antiapoptotic Treatment

Programmed cell death plays an important role in the regulation of cardiovascular homeostasis. Apoptosis contributes to cardiomyocyte loss in patients with coronary ischemia and HF, participates in ventricular remodeling in patients with previous MI, and is strengthened by neurohormone activation, thus participating in HF progression. In patients with ischemic heart disease or HF, the cellular values of proapoptotic proteins ([Bax, Bak, Bcl-xS/L, FasL], mitochondrial cytochrome c, and caspases 3 and 9) increase, whereas those of antiapoptotic proteins (Bcl2, BclxL, BclB, Bclw) decrease. The caspases also render some antiapoptotic proteins (Bcl, BclxL) inactive and even convert them into proapoptotic fragments. These findings suggest that cardiac apoptosis plays an important role in HF progression and that antiapoptotic therapy could save viable myocytes in HF patients. The overexpression of Bcl2 and suppression of the Bax (BclxL) subfamily increase cardiac survival, whereas caspase-3 activation increases apoptosis and its inhibition reduces apoptosis during ischemia. Similarly, FasL inhibition reduces cardiac apoptosis in ischemia models. In animal models endogenous caspase inhibitor analogues (FLIP-caspase 8, IAP-caspase proteins 3 and 9, zVAD-caspases 8 and 10) have been used. The stimulation of  $\beta$ -receptors increases cardiac apoptosis, which could explain the increase in mortality produced by  $\beta$ -adrenergic agonists in HF patients. On the other hand, statins, ACEI, and some ARA-II (candesartan) and beta-blockers (carvedilol, which increases Bcl-2 expression) inhibit cardiac apoptosis produced by inflammatory mediators, cytokines, and free radicals, which could be the reason why all these drugs reduce mortality in HF patients. The ischemic conditioning phenomenon produced by agonists of the mitochondrial potassium channels regulated by ATP has been attributed to their ability to eliminate changes in mitochondrial potential and cardiac apoptosis.<sup>123</sup> Nevertheless, before proposing antiapoptotic therapy in the treatment of cardiovascular disease, we should understand better the role of apoptosis in the genesis of cardiovascular disease and any consequences their inhibition could lead to in the long term.

### ALTERATIONS IN RELAXATION

A high percentage of HF patients have preserved

**TABLE 3. Clinical Trials in Diastolic Heart Failure\***

Trial	Inclusion	Criteria Aims	Duration	Drug
CHARM	CHF; EF>40%	Mortality Hospitalization	3 years	Candesartan Placebo
Wake Forest	EF>50%, AHT	Exercise tolerance, VO <sub>2</sub> max.	6 months	Losartan Hydrochlorothiazide
MCC-135	EF>40%	Exercise, remodeling		MCC-135 Placebo
I-PRESERVE	EF>45%	Mortality	2 years Total	Irbesartan Placebo

\*EF indicates ejection fraction; AHT, arterial hypertension; CHF, congestive heart failure.

systolic ventricular function. This situation is especially frequent in elderly patients with left ventricular hypertrophy, and manifests as lung congestion symptoms. In theory, the ideal drug should modify the mechanisms that cause diastolic dysfunction. These mechanisms should include normalization of calcium homeostasis and cardiac energy, suppression of neurohormonal activation, and/or the prevention or delay of fibrosis.<sup>124-126</sup> However, with some exceptions, there is a lack of studies that have investigated the effect of drugs on diastolic dysfunction. The reasons for this include failure to recognize the importance of diastolic dysfunction, the heterogeneity of the population studied and the absence of an accepted definition and diagnostic criteria for diastolic HF. The CHARM study (Candesartan in Heart failure Assessment of Reduction in Mortality and morbidity) (CHARM-Preserved) is the only suitably designed clinical trial to analyze the clinical efficacy of candesartan in HF patients (functional class II–IV) with preserved EF (>40%). It was demonstrated that candesartan does not modify the incidence of cardiovascular death, but it does reduce hospitalization due to HF ( $P=.01$ ).<sup>79</sup>

The effect of various drugs on patients with diastolic dysfunction or HF with preserved left ventricular function is being currently analyzed (Table 3). The PEP-CHF study (Perindopril in Elderly People with Chronic Heart Failure), included over 1000 patients more than 70 years old without systolic dysfunction (echocardiographic EF<40% or parietal movement index <1.4).<sup>127</sup> The I-PRESERVE study (Irbesartan in Heart Failure with Preserved Systolic Function) included patients with EF≥45% and excluded those presenting systolic HF. The SENIORS substudy (Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalization in heart failure) included patients over 70 years old with heart disease and EF>35%. Another study is investigating the effects of modulating calcium homeostasis using MCC-135, a drug that increases Ca reuptake in the sarcoplasmic reticulum and inhibits the cardiac cell membrane Na-Ca exchanger; as a consequence, intracellular [Ca]<sub>i</sub> is reduced

and cardiac relaxation improved. Two other small studies are using echocardiographic criteria to identify diastolic HF patients.

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