Direct Effects of 3 Combinations of Enalapril, Metoprolol, and Spironolactone on Cardiac Remodeling in Dilated Cardiomyopathic Hamsters

BRUNO LAVIOLLE, MD,[1] DANIELLE PAPE, PhD,[1] BRUNO TURLIN, MD, PhD,[2] AND ERIC BELLISSANT, MD, PhD[1]

Rennes, France

ABSTRACT

Background: In congestive heart failure, enalapril (E), metoprolol (M), and spironolactone (S) improve survival. Their direct effects on cardiac remodeling, when administered in combination, remain to be assessed.

Methods and Results: One hundred and five cardiomyopathic hamsters were divided into 5 groups (control, E, E-M, E-S, E-M-S) and treated from 150 to 240 days of age (E: 3 mg/kg, M: 1 mg/kg, S: 20 mg/kg). Cardiac and systemic hemodynamics, and cardiac remodeling were investigated. There was no difference between groups on blood pressure. Compared with C, both S-treated groups significantly increased cardiac index (E-S: +49%; E-M-S: +46%). Compared with C, E significantly decreased left ventricular (LV) cavity area (−10%). Compared with E, all combinations significantly decreased LV cavity area (E-M: −13%, E-S: −22%, E-M-S: −19%) and right ventricular (RV) collagen density (E-M: −18%, E-S: −30%, E-M-S: −34%), and the tri-therapy significantly decreased LV collagen density (−18%). Compared with bi-therapies, the tri-therapy significantly decreased LV (−19% vs. E-M, −16% vs. E-S) and RV (−20% vs. E-M) collagen densities.

Conclusion: At subdepressor doses, both bi-therapies induced similar effects on myocardial remodeling, enhancing the effects of E on LV cavity area and reducing RV collagen density. Compared with bi-therapies, the tri-therapy induced additional effects on LV and RV collagen densities. (*J Cardiac Fail 2006;12:752−758*)

Key Words: Adrenergic beta-antagonists, Aldosterone antagonists, Angiotensin-converting enzyme inhibitors, Heart failure.

Congestive heart failure (CHF) is a complex clinical syndrome characterized by neurohormonal activation and ventricular remodeling. The renin-angiotensin-aldosterone system and the sympathetic nervous system play an important role in the progression of cardiac dysfunction.[1,2] Drugs that can block their activations such as angiotensin I-converting enzyme (ACE) inhibitors,[3,4] β-blockers,[5,6] aldosterone antagonists,[7] and angiotensin II type 1 receptor antagonists[8] have been successively demonstrated to improve symptoms and cardiac function, and to decrease morbidity and mortality in patients with mild-to-severe CHF. These beneficial effects have been mostly related to the decrease of cardiac workload, but there is now growing evidence for a direct effect of these drugs on cardiac remodeling. Several mechanisms have been highlighted such as the inhibition of metalloproteinases for ACE inhibitors,[9] reduction of the catecholaminergic neuronal activity for β-blockers,[10] and the reduction of cardiac fibrosis for aldosterone antagonists.[11] However, the direct effects on cardiac remodeling when these drugs are given in combination remain to be explored.

In 2 previous experiments performed in a model of dilated cardiomyopathy, we found that the combination of metoprolol and spironolactone[12] and the combination of enalapril and spironolactone[13] induced stronger effects than each drug alone on cardiac remodeling. However, all animals received hydrochlorothiazide, and both metoprolol[12] and

From the [1]Laboratoire de Pharmacologie Expérimentale et Clinique, UPRES EA 3194 Groupe de Recherche Cardio-Vasculaire, Faculté de Médecine, Rennes, France; and [2]Département d'Anatomie et Cytologie Pathologiques, Centre Hospitalier Universitaire, Hôpital de Pontchaillou, Rennes, France.

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Reprint requests: Eric Bellissant, MD, PhD, Laboratoire de Pharmacologie Expérimentale et Clinique, Faculté de Médecine, 2, Avenue du Professeur Léon Bernard, CS 34317, 35043 Rennes Cedex (France).

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enalapril[13] were administered at hypotensive doses. We, therefore, made a new experiment to assess the effects of these drugs on cardiac remodeling when they are administered in combination at subdepressor doses. A tri-therapy with enalapril, metoprolol, and spironolactone was compared with 2 bi-therapies, one with enalapril and metoprolol, the other with enalapril and spironolactone, and to a monotherapy with enalapril.

Methods

Animals and Treatments

One hundred and five cardiomyopathic male hamsters (Bio TO-2 dilated strain) where obtained from Bio Breeders Inc. (Fitchburg, Massachusetts). The animals where housed individually under identical conditions with 12-hour light/dark cycles, free access to chow and water, and a room temperature of 21°C. At 140 days of age, they were randomly assigned to 1 of the 5 following groups (21 animals per group): control (C), enalapril alone (E), enalapril + metoprolol (E-M), enalapril + spironolactone (E-S), enalapril + metoprolol + spironolactone (E-M-S).

Animals were treated from 150 days to 240 days of age by gastric gavage every morning. Treatments were suspended in 5% gum arabic (vehicle). Hamsters were weighed every week in order to adjust drug doses. The control group was administered .5 mL/100 g body weight of the vehicle. Enalapril, metoprolol, and spironolactone were obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France). Daily doses were enalapril: 3 mg/kg, metoprolol: 1 mg/kg, and spironolactone: 20 mg/kg. These doses were the lowest found in the literature to improve cardiac remodeling without inducing any effect on systemic blood pressure in cardiomyopathic hamsters.[10,13,14]

All experiments were conducted in accordance with the national guidelines for the use of laboratory animals (approval number 006515 given by the French Ministry of Agriculture).

Investigated Variables

Hemodynamics. The following hemodynamic variables were processed in anesthetized animals using specialized software (IOX; EMKA Technologies, Paris, France). Left ventricle (LV) and systemic blood pressures were measured using a 2-French micromanometer-tipped catheter (SPR 407; Millar Instruments Inc., Houston, Texas). LV systolic and end-diastolic pressures (mm Hg), maximum positive and negative dP/dt (±dP/dtmax; mm Hg/s) and time constant of relaxation (tau, ms) were derived from the LV pressure signal. Systolic and diastolic blood pressures (mm Hg), and heart rate (beats/min) were derived from the systemic blood pressure signal. Blood flows were measured by the transit time method using calibrated perivascular flow probes connected to an ultrasonic flowmeter (Model T206 Dual Channel; Transonic Systems, Ithaca, New York) as previously described.[15] Cardiac output (mL/min), mesenteric and renal blood flows (mL/min) were derived from the signals measured at the level of the abdominal aorta (just below the diaphragm), the mesenteric artery (first order), and the right renal artery, respectively. Mean blood pressure (mm Hg), stroke volume (mL), cardiac index (cardiac output normalized by body weight, mL/min/g), systemic vascular resistances index (systemic vascular resistances normalized by body weight, mm Hg.min/mL/g), and mesenteric and renal vascular resistances (mm Hg.min/mL) were calculated.

Morphometry and Histomorphometry. Animals (g) and target organs (mg) — whole heart, atria, LV, right ventricle (RV), lungs, and liver — were weighed. Each organ weight was normalized by body weight. Histomorphometric analyses were performed using a computer-assisted image analyzer (Histolab and Saisam; Microvision Instrument, Evry, France) as previously described.[15]

Heart. LV wall thickness (mm), LV cavity area (mm^2), and LV and RV collagen density (%) were assessed on Sirius red-stained slides. In each case, 15 measures were performed to determine a mean value.

Lungs and Liver. Pulmonary and hepatic congestions were estimated using arbitrary scales as previously described.[15]

Experimental Protocol

Animals were investigated in a random manner. All variables were measured on the same animal and similarly, whichever the group.

Just before in vivo investigation, hamsters were anesthetized with intraperitoneal injection of ketamine (200 mg/kg) and xylazine (5 mg/kg). Lungs were ventilated using a rodent respirator with a tidal volume of 1.6 mL, and at rates ranging from 60 to 70/min.

Hemodynamics. The right carotid artery was cannulated, and the catheter was advanced into the aorta. After a 5-minute stabilization period, systemic blood pressure signal was recorded. Thereafter, the catheter was advanced into the LV to record LV pressure. Afterwards, a laparotomy was performed to isolate the abdominal aorta and the mesenteric and renal arteries. A perivascular flow probe (1.5 R) was placed around the aorta to record cardiac output. Hence, another perivascular flow probe (.5 V) was placed successively around the mesenteric and renal arteries to record corresponding blood flows. All variables were recorded during 30 seconds.

Morphometry and Histomorphometry. After exsanguination, the heart, the lungs, and the liver were prepared for light microscopy as previously described.[15]

Statistical Analysis

Reported values are expressed as means ± standard deviation for quantitative variables or numbers and percentages for qualitative variables. Statistical analysis was performed with SAS statistical software V8.02 (SAS Institute, Cary, North Carolina). For quantitative variables, between groups comparisons were performed using 1-way analysis of variance. In case of a statistically significant difference, pair-wise comparisons were performed using the Duncan multiple range test. For qualitative variables, between group comparisons were performed using the chi-square test. In each case, a P value $<.05$ was considered statistically significant.

Results

Three animals spontaneously died during the treatment period: 2 in the E group at 184 and 204 days of age and 1 in the E-S group at 151 days of age.