Effects of Bisoprolol and Losartan Treatment in the Hypertrophic and Failing Right Heart

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ABSTRACT

Background: Sympathetic adrenergic stimulation and the renin-angiotensin-aldosterone system are highly elevated in right heart failure. We evaluated if treatment with the adrenergic receptor blocker bisoprolol or the angiotensin II receptor blocker losartan could prevent the progression of right ventricular (RV) hypertrophy and failure in rats after pulmonary trunk banding (PTB).

Methods and Results: Male Wistar rats were randomized to severe PTB with a 0.5-mm banding clip (PTB0.5, n = 29), moderate PTB with a 0.6-mm banding clip (PTB0.6, n = 28), or sham operation (SHAM, n = 13). The PTB0.5 and PTB0.6 rats were randomized to 6 weeks of 10 mg/kg/d bisoprolol treatment, 20 mg/kg/d losartan treatment, or vehicle treatment. The PTB caused hypertrophy, dilation, and reduced function of the RV in all rats subjected to the procedure. Rats subjected to the more severe banding developed decompensated RV failure with extracardiac manifestations. Treatment with bisoprolol slowed the heart rate, and treatment with losartan lowered mean arterial pressure, confirming adequate dosing, but none of the treatments improved RV function or arrested the progression of RV hypertrophy and failure compared with vehicle.

Conclusions: In our PTB model of pressure overload−induced RV hypertrophy and failure, treatment with bisoprolol and losartan did not demonstrate any beneficial effects in compensated or decompensated RV failure. (*J Cardiac Fail 2014;20:864−873*)

Key Words: Right ventricular dysfunction, pulmonary hypertension, beta-adrenergic receptor blocker, angiotensin II receptor blocker.

Right heart failure (RHF) induced by pressure overload is an inevitable consequence of pulmonary arterial hypertension (PAH) and several types of congenital heart disease. The normal right ventricle (RV) is a thin-walled and highly compliant chamber that tolerates high pressures poorly. In PAH and congenital heart diseases, the afterload is increased due to progressive vascular remodelling, an RV supporting the systemic circulation, or an anatomically obstructed RV. To maintain cardiac output, the RV hypertrophies. However, the sustained and increasing pressure overload ultimately leads to deterioration of RV function and development of RHF.[1] RV function is the most important determinant of prognosis and death in PAH-patients.[2] Current PAH treatments aim to reduce RV afterload and thereby secondarily improve RV function to some extent.[3] But even with optimal treatment and a subsequent decrease in pulmonary vascular resistance, most patients still develop RHF,[4] and there is currently no available treatment that directly inhibits pathologic remodeling of the RV.

Inhibition of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS) by angiotensin II receptor blockers (ARBs) and β-blockers improve cardiac function and survival[5,6] and are key therapies in the treatment of left heart failure. We know that RAAS and SNS activity are increased in PAH and RHF.[7,8] The increase in SNS activity is an independent predictor of clinical deterioration in PAH,[9] and hyponatremia (an indirect

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indicator of RAAS activity) is associated with RHF and poor survival in PAH.[10] Despite this and the well documented beneficial effects in patients with left heart failure, neither β-blockers nor ARBs are used routinely in treatment of patients with RHF.

Preclinical studies of β-blocking and ARB treatments have mainly been conducted in animal models of nonfixed afterloads, making it difficult to distinguish direct cardiac effects from afterload reducing pulmonary effects.[7,11–13] Studies investigating ARB treatments in banding models have shown conflicting results.[14,15] A recent study evaluating the effects of combination therapy with losartan and eplerenone in a rat model of pulmonary artery banding demonstrated no beneficial effects of the treatment on RV function or remodeling.[16] The studies investigating monotherapy of ARB were conducted in pulmonary artery banding models of compensated RV hypertrophy and not decompensated RHF with extracardiac manifestations. In left heart failure, effects of treatment may depend on the magnitude of pressure overload[17]; therefore, effects of ARBs may be different in compensated and decompensated RHF.

Bisoprolol is a β_1-specific and thereby cardiac-specific adrenergic receptor blocker. Losartan is an ARB specific for the AT_1 receptor. In contrast to treatment with angiotensin-converting enzyme (ACE) inhibitors, treatment with losartan does not negate the potential cardioprotective effects of the AT_2 receptor.[18] The aim of the present study was to evaluate the direct cardiac effects of treatment with bisoprolol and losartan in a rat model of pressure overload–induced compensated and decompensated RHF caused by pulmonary trunk banding (PTB).

Methods

Animals

Male Wistar Galas rats (M&B Taconic, Ry, Denmark) (105 ± 30 g; n = 70) were given free access to tap water and standard rat chow (Altromin #1324; Altromin, Lage, Germany) and housed in a room with a 12-hour light-dark cycle and a temperature of 23°C. The rats were treated according to Danish national guidelines, and all experiments were approved by the Institutional Ethics Review Board and conducted in accordance with the Danish law for animal research (authorization number 2012-15-2934-00384, Danish Ministry of Justice).

Study Design

Rats were randomized to PTB with a diameter of 0.6 mm (PTB0.6; n = 28), 0.5 mm (PTB0.5; n = 29), or sham surgery (SHAM; n = 13) (Fig. 1). One week after the procedure, a subset of animals from each group, SHAM (n = 6), PTB0.6 (n = 6), and PTB0.5 (n = 6), were evaluated to assess cardiac function at the onset of treatment, and the remaining PTB rats were subjected to echocardiography and randomized to treatment with 20 mg/kg/d losartan (Losarstad, Stada Arzneimittel) (PTB0.6: n = 9; PTB0.5: n = 9), 10 mg/kg/d bisoprolol (Stada Arzneimittel) (PTB0.6: n = 8; PTB0.5: n = 9), or vehicle (PTB0.6: n = 11; PTB0.5: n = 11) by once-daily oral gavage. Dosages were chosen based on previously conducted dose-finding studies with telemetric evaluation of hemodynamics in awake rats during normal activity. The 20 mg/kg/d dosage of losartan was the maximal tolerated dosage causing <10% drop in aortic pressure.[7] The 10 mg/kg/d dosage of bisoprolol was the minimal dosage that was able to completely blunt heart rate (HR) response during daily activity.[13] The SHAM rats received vehicle treatment. After 6 weeks of treatment, the effects were evaluated by echocardiography, magnetic resonance imaging (MRI), and pressure/volume measurements. Blood samples were drawn, the animals killed, and anatomic measures and clinical signs of heart failure evaluated.

Pulmonary Trunk Banding

Banding of the pulmonary trunk was performed to induce RV pressure overload as described previously.[19] Rats were sedated, intubated, and mechanically ventilated with sevoflurane (Abbot Scandinavia, Solona, Sweden; induction: 7.0% in 2:1 O_2/N_2O mix; maintenance: 3.5% in 2:1 O_2/N_2O mix) at 75 breaths/min and a tidal volume of 10 mL/kg. A lateral thoracotomy was performed and the pulmonary trunk and the aorta carefully separated, enabling the appliance of a titanium clip with an inner diameter of 0.5 mm or 0.6 mm around the pulmonary trunk. To compensate for fluid loss and relieve postoperative pain, 3 mL isotonic saline solution and buprenorphine (Termgesic; RB Pharmaceuticals, Sough, United Kingdom) was administered subcutaneously (0.12 mg/kg) and in the drinking water 3 days after surgery (7.4 μg/mL). SHAM rats underwent the same procedure without applying the clip.

Evaluation of Hemodynamic and Anatomic Measures

Right ventricular dimensions and function were assessed with the use of imaging modalities—echocardiography and MRI—and invasive pressure-volume measurements. For details see the supplemental material available online. After drawing a blood sample from the abdominal aorta for hemodynamic evaluation, we euthanized the rats with the use of full anesthesia (details above). The organs were weighed (Sartorius, Göttingen, Germany), the length of the tibia measured, and the RV weight divided by tibia length used as a measure of RV hypertrophy. The presence of ascites and hydrothorax was estimated with the use of cotton swabs weighed before and after wiping the abdominal and pleural cavity. A surplus of >1 g was used as a cutoff. The presence of nutmeg liver was confirmed as a dark discoloration of the liver. RV free wall tissue was kept in formalin for histology and snap frozen for molecular analyses. Methods for histology and quantitative polymerase chain reaction for determination of A-type natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and β-myosin heavy chain (MHC) expression are described in the online supplement.

Statistics

Unless otherwise stated, normally distributed quantitative data are expressed as mean ± standard error of mean (SEM) and nonnormal data as mean with 95% confidence interval (CI). All statistical analyses were performed with the use of Graphpad Prism 6 (Graphpad Software, La Jolla, California). Data were tested for normal distribution with the use of a Shapiro-Wilk normality test, and nonparametric tests were used if not normally distributed. One-way analysis of variance was used for evaluation of significance of differences, followed by post hoc Bonferroni analyses of selected groups to evaluate the model (PTB0.5 + vehicle vs PTB0.6 + vehicle, and both vs SHAM) and the effects of treatments