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ANIMAL AND IN VITRO MODELS IN HUMAN DISEASES

The remodeling of connexin in the hypertrophied right ventricular in pulmonary arterial hypertension and the effect of a dual ET receptor antagonist (bosentan) ☆

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Abstract

Studies on the role of connexins (Cxs) in the pathogenesis of right ventricular (RV) hypertrophy in pulmonary arterial hypertension (PAH) have not been reported to date. Therefore, we established a rat model of PAH induced by monocrotaline (MCT), and they were randomized to three groups: Control, MCT, and MCT + bosentan. Through electromicroscopy, in the control group, the gap junctions were long and frequent in intercalated disks, and short and rare at the sites of side–side cell junctions. In the MCT group, the opposite distribution was detected. In the MCT + bosentan group, the distribution of gap junctions was similar to that in the control group. Using immunoconfocal microscopy, most of the Cx43 staining was aggregated at the cell termini, and staining was weak at the sites of side–side cell junctions in the control group. However, the distribution of Cx43 was opposite in the MCT group. In the MCT + bosentan group, the result was similar to that in the control group. Therefore, perturbation of connexin distribution may be associated with RV hypertrophy. Improving the distribution of Cx43 in RV myocardium may be one of the mechanisms of a dual ET receptor antagonist partly reversing the RV hypertrophy.

Keywords: Pulmonary arterial hypertension; Connexin; RV myocardium; Remodeling

Introduction

Pulmonary arterial hypertension (PAH) is a heterogeneous group of disorders characterized by increased pulmonary artery pressure and pulmonary vascular resistance. Without intervention, PAH has a progressive downhill course, leading to right ventricular (RV) failure and death [1]. Therefore, the question of how to ameliorate RV remodeling and delay cardiac functional aggravation on the basis of improving the treatment of PAH has become very important.

Abbreviations: Cx, connexin; PAH, pulmonary arterial hypertension; RV, right ventricle; LV, left ventricle; ET, endothelin; MCT, monocrotaline; PFA, paraformaldehyde; HE, hematoxylin and eosin; PTH, Mallory phosphotungstic acid–hematoxylin; BSA, bovine serum albumin; GJ, gap junction.

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Gap junctions (GJ) are cell membrane protein channels clustered at cell–cell junctions, allowing the exchange of ions and small secondary messenger molecules, such as calcium, nucleotides, and inositol triphosphate [2], which forms matabolic coupling. Meanwhile, gap junctions mediate electrical coupling. Intercellular communication through gap junctions plays an important role in the maintenance of the normal function of organs, embryonic differentiation, and growth control in multicellular organisms. Each gap junction channel is formed by an interconnection of hemichannels consisting of hexamer subunit proteins called connexin (Cx) [3].

Gap junctions are dynamic structures. Connexin expression and distribution will be variable when the tissue is impaired, which is termed remodeling of gap junctions [4]. Recent studies have demonstrated that remodeling of gap junctions plays an important role in the occurrence and development of many diseases, such as coronary heart disease, cardiac arrhythmias [5–10], tumors [11,12], and ventricular hypertrophy [13]. However, studies dealing with the role of connexin in the pathogenesis of RV remodeling in PAH have not yet been reported.

The aim of this study was to investigate changes of Cx43 expression and distribution in the RV myocytes of rats with monocrotaline (MCT)-induced PAH after bosentan (a dual endothelin (ET) receptor antagonist) treatment in order to provide new ideas for improving RV remodeling. These results may have important clinical implications.

Materials and methods

Animals

Male Sprague Dawley rats, weighing between 150 and 200 g, received intraperitoneal (IP) injection of either 0.5 ml 0.9% saline or 0.5 ml 60 mg/kg MCT (Sigma Chemical Co. USA). Two weeks later, they were randomly assigned to three groups receiving oral therapy with vehicle (gummi arabicum 5%) or bosentan (300 mg/kg/day, ACTELION, Switzerland) over a 3-week period: control group ($n = 10$), MCT ($n = 35$), and MCT + bosentan ($n = 22$).

Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Research Committee at Fu wai Hospital in China.

Experimental protocol

Forty-eight hours after active treatment was stopped, surviving rats were anesthetized with pentobarbital sodium (50 mg/kg, ip), followed by 2000 U heparin. Administration of study drugs was stopped 48 h before hemodynamic measurements to evaluate the chronic effect of therapy with no active medications in plasma. After stable anesthesia was obtained, a polyethylene catheter (PE50; 0.97 mm OD, 0.58 mm ID) connected to a pressure transducer was inserted into the right carotid artery to measure arterial blood pressure recorded by polygraph system (RM-6300, Nihon Koden, Tokyo, Japan). Another polyethylene catheter was inserted into the right jugular vein to measure RV pressures. Blood pressure traces identified the intravascular location of the catheter tips. The heart was excised and used for morphometric analysis.

Histological studies

Macroscopic morphology of heart – estimation of right ventricular hypertrophy

Transverse sections of the heart just inferior to the mitral valve leaflet were evaluated for RV-free wall and left ventricle (LV)-free wall thickness using a reticle eyepiece at 40 × magnification. The calculated RV/LV ratio was considered an index of RV hypertrophy.

Examination through light microscopy

Blocks (1 cm × 0.3 cm) were taken from the RV-free wall and fixed in 4% paraformaldehyde (PFA) for 24 h at 4 °C, followed by fixation in paraffin. Tissue was cut in sections (3 μm) and stained with the hematoxylin and eosin (HE) or Mallory phosphotungstic acid-hematoxylin (PTH) technique.

Examination through electron microscopy

Blocks (1.5 mm × 1 mm × 1 mm) were taken quickly from the RV-free wall and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, PH 7.4, at room temperature for 7 h. Specimens were postfixed with 1% osmium tetraoxide in 0.1 M cacodylate buffer and stained *en bloc* with 4% uranyl acetate at room temperature before embedding in Epon 812. Ultrathin sections were double stained with uranyl acetate and lead citrate, and observed through a JEOL 2000 EX electron microscope (Tokyo, Japan) at an accelerating voltage of 100 kV. Morphometric analysis was carried out using the method of Luke and Saffitz [14]. Five randomly selected fields in longitudinally sectioned ventricular tissue blocks were analyzed in each rat.

All portions of each test area containing intercalated disks and gap junction profiles were rephotographed for further analysis at a final print magnification of × 24,000. Intercalated disk and gap junction profile lengths were measured in each high-power micrograph using an electronic stylus and digitizing tablet (Houston