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Original Research

Tissue Doppler Imaging and Two-dimensional Speckle Tracking of Left Ventricular Function in Horses Affected with Recurrent Airway Obstruction before and after Clenbuterol Treatment

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ABSTRACT

Cardiac effects of the β_2-adrenergic agent clenbuterol have been the focus of many studies, but effects on myocardial velocities and myocardial deformation parameters have not yet been evaluated in horses affected with recurrent airway obstruction (RAO) using tissue Doppler imaging (TDI) and two-dimensional speckle tracking (2DST). In our study, 7 horses affected by RAO were treated over 14 days with clenbuterol, 0.8 μg/kg every 12 hours. Standard echocardiographic, TDI (pulsed wave and color TDI), and 2DST examinations were performed before and after the treatment period. Myocardial function was recorded in the right parasternal short-axis view. Percent of fractional shortening and two-dimensional echocardiography (2DE) measurements did not show any significant changes after 2 weeks of treatment. Early diastolic velocity, *E*, increased significantly after clenbuterol in the left ventricular free wall (LVFW; $P = .001$). The *E*/late diastolic velocity (*A*) quotient ($P = .003$) and the isovolumetric contractility ($P = .035$) also increased significantly after treatment. Time parameters, particularly the time interval between the Q-wave in the echocardiograph and atrial release, the time of diastole and Tei index (parameter of global ventricular function), decreased significantly after clenbuterol administration in the LVFW ($P = .014$/$P = .028$/$P = .015$, respectively). The 2DE speckle tracking revealed a significant increase of the early diastolic systolic strain rate ($P = .01$) in the LVFW after therapy. In conclusion, 2 weeks of treatment with clenbuterol at a dosage of 0.8 μg/kg every 12 hours led to improved cardiac function in severely RAO-affected horses. This could be a sign of myocardial restoration (re-remodeling) after therapy.

1. Introduction

Clenbuterol is used as a broncholytic agent in respiratory disease that causes bronchospasm, for example, in subacute and chronic bronchitis and bronchiolitis, in recurrent airway obstruction (RAO) and as a supportive drug in acute bronchitis and bronchopneumonia [1,2]. In addition to its bronchodilatory effects, clenbuterol is known for its anabolic effects on fat and muscle tissue repartitioning in cardiac and skeletal muscle [3-5]. The effects of ß$_2$-adrenergic agents on skeletal muscle and cardiac function in particular have been discussed intensively, and there has been a lot of controversy about its negative and positive cardiac effects [6,7]. The combined use of pharmaceutical agents such as clenbuterol for the treatment of congestive heart failure in men is regarded as an important research focus. There are changes at molecular, cellular, biochemical, and structural levels in the myocardium over the course of ventricular dysfunction, which lead to changes in myocardial geometry and architecture, which is called remodeling [8-10]. This remodeling is characterized by hypertrophy of the cardiomyocytes, leading to a dysfunction of the cells' contractility [11]. As

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cardiac remodeling is a bidirectional process, reverse remodeling from a pathological state to a physiological state is possible [12]. Physiological cardiac hypertrophy is achieved in humans by the use of clenbuterol in a dosage of up to 700 μg every 8 hours, comparable to the effects of intense exercise [13]. This increase in ventricular function and decrease in wall tension allows the recovery of the cardiac system [14]. Application of a selective ß$_1$-blocker during clenbuterol treatment reduces the negative side effects of ß$_1$ stimulation, including not only the increased heart frequency and blood pressure but also pathologic cardiac hypertrophy characterized by fibrosis [15].

In contrast to this, several studies have found a reduction in cardiac function and a potential risk of cardiomyopathy after clenbuterol application [16-19]. In addition to necrosis and apoptosis in the musculus soleus, plantaris, and tibialis anterior muscles, necrosis, particularly in the subendocardium of the left ventricle apex, was found in rats [15,16]. Burniston et al [17] reported a dose dependence, which allows the separation of physiological cardiac hypertrophy and pathological myotoxic effects. Echocardiographic and histologic studies in mice found functional and structural improvements at a dosage of 2.4 μg/kg over 7 days [20]. Negative effects after clenbuterol application have also been described in horses. The aortic diameter was increased by 24% to 39% after 8 weeks of clenbuterol treatment at a dosage of 2.4 μg/kg every 12 hours, particularly after exercise, and was interpreted as an increased risk of aortic root rupture [18].

The effect of clenbuterol on myocardial velocities and myocardial deformation parameters in healthy horses has been examined before. After clenbuterol application of 0.8 μg/kg every 12 hours over 14 days, an improvement in diastolic function and improved relaxation characteristics of the cardiac muscle could be demonstrated [21]. In the present study, the cardiac effects of clenbuterol were evaluated by tissue color Doppler imaging (TDI) and two-dimensional speckle tracking (2DST) in RAO patients without additional cardiac disease. TDI offers the ability to measure different myocardial velocities, and 2DST offers the possibility of measuring myocardial deformation parameters. Both tests allow a more detailed quantification of global and regional myocardial functions. The reliability of both techniques in horses has been described previously [22-27].

2. Materials and Methods

2.1. Animals

The study population consisted of 7 Warmblood horses presented to the Equine Clinic of the University of Munich with a history of RAO over several years.

The 5 geldings and 2 mares were between the ages of 13 and 23 years old (mean age, 17 ± 4; mean height, 164 ± 7 cm; mean weight, 581 ± 40 kg).

2.2. Preparticipation Examination

After detailed clinical examination of the horses and questioning of the owners regarding the history of the horse, we performed specific examinations of the cardiovascular system and physical examination of the respiratory system, including auscultation and percussion. Horses were eligible for participation in the study provided that the owner gave informed consent. Horses were ineligible for inclusion in the study if they had clinical signs of additional heart disease or had been given any kind of premedication up to 8 weeks previously.

2.3. Lung Examination

The lung examination included history, clinical lung auscultation, arterial blood gas analysis, endoscopy, cytological evaluation of tracheobronchial secretions, and radiography.

2.4. Echocardiographic Examination and Off-line Data Analysis

Echocardiographic examinations and off-line analyses were performed by one experienced observer throughout the entire study period, using a Vivid *i* echocardiography unit[a] with continuous base–apex electrocardiography (ECG). A 3.5-MHz annular phased-array probe[b] with a maximum depth of 30 cm was used.

Standardized right and left parasternal B-mode and color flow Doppler images were recorded for evaluation of cardiac dimensions and valvular functions. A right parasternal short axis (SAX) M-mode image of the left ventricle was acquired at mitral valve level for the calculation of percentage of fractional shortening (FS%).

A right parasternal SAX image of the left ventricle was acquired at the chordal level immediately below the mitral valve for TDI and 2DST. A depth of 25 cm and an image angle of 65° were chosen. Using these settings as a preset, we achieved a frame rate of at least 63.3 frames per second with sufficient image quality.

2.4.1. Color TDI

After identification of the correct echocardiographic position, B-mode was switched to color TDI mode, and three rhythmic and artifact-free cardiac cycles were recorded, as follows. Analysis of myocardial velocities and time parameters were performed offline using EchoPAC software.[c] Therefore, the regions of interest (ROI) within 10 × 10 mm were positioned close to the endocardium in the different myocardial regions (left ventricular free wall [LVFW] and interventricular septum [IVS]). ROIs were manually tracked over the entire cardiac cycle to ensure an appropriate position within the myocardium. In addition to several myocardial velocities (systolic [S] peak velocity, early [*E*] diastolic velocity, and late diastolic velocity [*A*]), different time parameters also were measured (isovolumetric contractility time [IVCT]; isovolumetric relaxation time [IVRT]; time interval between the Q-wave of the

[a] Vivid *i* echocardiograph, General Electric Healthcare, Medical Sy2DSTms (application software version 6.1.110; Sy2DSTm software version 1.36.18).

[b] Vivid *i*, 3s-RS probe, General Electric Medical Sy2DSTms.

[c] Version 7.0 EchoPac software only; Firma GE Healthcare, Horton, Norway.