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Chronic electroacupuncture of the ST36 point improves baroreflex function and haemodynamic parameters in heart failure rats

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ABSTRACT

Electroacupuncture (EA) has been used to treat many diseases, including heart failure (HF). This study aimed to evaluate the effects of chronic stimulation in the ST36 acupuncture point on haemodynamic parameters and baroreflex function in rats with HF. Cardiovascular parameters assessed were heart rate (HR), blood pressure (BP), and the reflex cardiovascular response of HR triggered by stimulation of baroreceptors in animals with HF subsequent to acute myocardial infarction (AMI). Male Wistar rats were divided into three groups: Sham Control — animals without HF and without EA; HF Control group — animals with HF and without EA; and HF EA group — animals with HF that received the EA protocol. Six weeks after surgical induction of AMI, the EA protocol (8 weeks, 5 times a week) was performed. The protocol was applied with EA at the ST36 point, frequency of 2 Hz, pulse of 0.3 ms and intensity of 1–3 mA for 30 min. Haemodynamic parameters and baroreceptor function were assessed. There was no difference between groups in the variables HR, systolic blood pressure (SBP) and diastolic blood pressure (DBP), which were evaluated with awake animals ($p > 0.05$). There was an increase in the mean arterial pressure (MAP) in the HF EA group compared to the HF Control group ($p < 0.05$). The maximum gain of the baroreflex heart rate response (Gain) was higher in the HF EA group than the HF Control and Sham Control groups. Chronic EA in the ST36 point increased the MAP and baroreflex sensitivity in rats with HF.

1. Introduction

Heart failure (HF) is a progressive syndrome in which a structural or functional cardiac condition impairs the heart's ability to adequately supply blood flow to the various organs and tissues (Axente et al., 2011). Acute myocardial infarction (AMI) is the most common etiologic factor for the development of HF (von Haehling et al., 2009). Initially, there is an increase in sympathetic cardiac activity in order to maintain the cardiac output (CO). The most important symptoms that appear in the early stage of HF are dyspnoea and exercise intolerance (Antunes-Correa et al., 2010).

However, the maintenance of this sympathetic activation leads to deleterious effects throughout the cardiovascular system (Jaenisch et al., 2011). In this sense, HF is characterised by an imbalance in the sympathetic–vagal physiological activity with a chronic adrenergic activation, together with impairment in the baroreflex sensitivity (La Rovere et al., 2008).

The baroreflex is one of the most important reflexes able to regulate the heart rate (HR) and blood pressure (BP) since there is a direct influence on the autonomic nervous system (Quagliotto et al., 2008). Baroreceptors are sensitive to mechanical deformations of the vascular walls and promote short-term blood pressure control. Changes in the characteristics of the baroreflex function, such as a reduction in the sensitivity due to hyperactivation of the sympathetic system, may alter the autonomic control of the cardiovascular system (La Rovere et al., 1998; Zhang and Anderson, 2014). Thus, the evaluation of the sensitivity of the baroreflex has become an important prognostic marker for cardiovascular disease (La Rovere et al., 2013) and cardiac death after acute myocardial infarction (La Rovere et al., 1998).

Experimental studies have been conducted to elucidate the effects of acupuncture on the cardiovascular system (Middlekauff, 2004; Wang et al., 2008; Li et al., 2012) and on the autonomic system function (Middlekauff et al., 2002; Sugimachi et al., 2007; Huang et al., 2011), including changes in the sympathetic and the parasympathetic activity and also in the baroreflex function (Middlekauff et al., 2002; Tsou et al., 2004; Sugimachi et al., 2007; Xiong et al., 2011). Studies conducted on animals may elucidate the mechanisms by which electroacupuncture or manual acupuncture is capable of generating bradycardia and decreased blood pressure (Uchida et al., 2007, 2008; Li and Longhurst, 2010; Zhou

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and Longhurst, 2012). Besides that, these studies have helped in the understanding of the acupuncture mechanisms, demonstrating the specificity of each acupuncture point (Choi et al., 2012), such as the stomach point 36 (ST36), which has an effect on the cardiovascular and autonomic system (Tjen et al., 2004; Uchida et al., 2007).

However, this evidence is based mostly in healthy animals and little is known about the effects of acupuncture in HF (Middlekauff, 2004). Recently, a well-design study demonstrated that EA attenuates sympathetic over activity, improves cardiac function and remodelling and reduces infarct size in HF rats and postulated EA as a novel and potentially useful therapy for treating HF (Ma et al., 2014). Furthermore, most studies show only short-period acupuncture applications, which makes it difficult to prove the long-lasting effects of acupuncture in chronic situations. Our hypothesis is that a protocol of chronic electroacupuncture is capable of improving baroreflex sensitivity in rats with heart failure. Therefore, the primary objective of this study was to evaluate the effect of chronic electroacupuncture in the ST36 point in baroreflex sensitivity, and a secondary objective was to evaluate the haemodynamic parameters of rats with heart failure.

2. Methods

2.1. Animals

Male Wistar rats ($n = 32$), weighing between 200 and 300 g (approximately 60 days old), from the animal breeding centre of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) were used. Animals were kept in a cage with ad libitum food and water in a room with a 12:12 h dark/light cycle. The study followed the Law 11.794 of 10/08/2008 and Decree No. 6.899 of 07/25/2009, which currently regulates research activities conducted with animals, as well as the guidelines contained in the International Guiding Principles for Biomedical Research Involving Animals from the Council for International Organizations of Medical Science (CIOMS). All procedures performed in this study were previously approved by the UFCSPA Ethics and Research Committee (Protocol 088-12).

2.2. Surgery to induce myocardial infarction

Rats were anaesthetised by inhalation of 2% isoflurane (Isoforine, 100 mL — Cristália) and 98% oxygen (Munkvik et al., 2011) and intubated, and anaesthesia was maintained at the same concentration of isoflurane through the tracheal tube with 100% oxygen and the use of a vaporizer with 1 L/min^{-1} flow of gas (rotometer for veterinary anaesthesia, BR-200 Vetcare, Incotec Científica LTDA, Brazil). Animals were maintained at Guedel's anaesthetic level III (Hecker et al., 1983; Colman et al., 2002). Myocardial infarction was induced as previously described (Pfeffer et al., 1979; Hentschke et al., 2013). The thoracotomy was performed at the level of the left fourth intercostal space, the pericardium was cut and the left anterior descending coronary artery was ligated with a 6.0 mono nylon wire. The chest incision was closed using 3.0 mono nylon wires, and the pneumothorax was drained by a continuous suction system. Then, the animals were removed from artificial ventilation and encouraged to breathe (Francis et al., 2004). As a prophylaxis against infections, single doses of penicillin (20,000 units) were administered. For the Sham Control group, the same procedures were conducted except for the performance of the coronary ligature.

2.3. Experimental groups

After the myocardial induction, the animals remained in the cages for 6 weeks (time necessary to develop the HF state). After this period, animals were assigned to one of three groups: Sham Control (Sham Control, $n = 9$), HF Control (HF Control, $n = 13$) and HF electroacupuncture (HF EA, $n = 10$).

2.4. Electroacupuncture protocol

Animals were adapted and gently handled for 30 min, for 2–3 days before the beginning of the protocol, always by the same researcher (Lao et al., 2004). For the EA protocol, acupuncture needles made of stainless steel, size 0.5 mm (Complementar Agulhas, Brazil), were inserted bilaterally in the stomach point 36, ST36 (ZuSanLi), which is located in the anterolateral portion of the hind limb, near the anterior tibial tuberosity, in the tibialis anterior muscle and is innervated by the deep fibular nerve (Tjen et al., 2004; Li et al., 2009). The needling was performed at a depth of 5 mm and confirmed by a slight muscle contraction (Wu et al., 2010) or by slight and repeated movement of the paw (Zhou et al., 2005). The NKL electro-stimulator (Model EL608) with eight channels was used. The parameters used are described in Table 1.

Animals were placed in a device developed in our laboratory for animal stimulation (unpublished data) but the control group didn't received EA: without needle insertion or electrical stimulation and stayed for the same 30-minute period (Fig. 1A).

2.5. Functional capacity test (VO_2 max)

After the completion of the EA protocol, animals were subjected to a functional capacity test. To determine maximum oxygen consumption (VO_2max), each animal accomplished the maximum testing capacity of Batista's adapted exercise (Batista et al., 2007). The parameters were measured using a gas analyser system for animals (AVS Projects, São Carlos, SP, Brazil). The air volume supplied was of 2.5 L/min. The analyser was calibrated with a mix of a known quantity of gas prior to each test. After a period of 15 min of acclimatisation, the test protocol was initiated at a speed of 10 m/min. The protocol consisted of a gradual increase in the speed of the treadmill with an increment of 5 m/min every 3 min. It was considered as exhaustive when the animal remained on the shock platform for more than 15 s.

2.6. Surgical preparation for cardiovascular measures on animals

After functional capacity test, animals were anaesthetised with ketamine (90 mg/kg ip) and xylazine (12 mg/kg ip). Two catheters filled with saline solution (NaCl 0.9%, pH 7.40, 0.6 mL) and heparin (0.01 mL) were inserted into the left femoral artery and vein to directly measure arterial blood pressure and for the administration of drugs, respectively. The concentration of heparin was 200 μg/mL heparin.

2.7. Baroreflex sensitivity

On the following day after vascular catheter implantation, the cannula was connected to an extension of 40 cm (PE-50) in order to enable the animal's movement. The arterial cannula was connected to a pressure transducer (Strain-Gauge, Narco Biosystem Miniature Pulse Transducer RP-155, Houston, Texas, USA) connected to a signal amplifier (Pressure Amplifier HP 8805C). The blood pressure signals were recorded for 15 min and were used as baseline data for each animal (CODAS — Data Acquisition System, PC 486). The “beat-to-beat” recorded data were

Table 1
Protocol parameters and electroacupuncture treatment scheme.

Electroacupuncture parameters	
Model device	NKL EL 608, manufacturer
Frequency	2 Hz
Pulse	0.3 ms
Intensity in the first 5′	1 mA
Intensity at 5 to 10′	2 mA
Intensity during 20′	3 mA
Intensity	1–3 mA
Total time	30 min
Duration of the treatment ST36 point	5× week/8 weeks