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Cardioprotective effects of isoflurane in a rat model of stress-induced cardiomyopathy (takotsubo)

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ABSTRACT

Background: Stress-induced cardiomyopathy (SIC) is a common syndrome with substantial morbidity and mortality. SIC is common in intensive care units' patients. No therapeutic intervention for SIC has been evaluated in randomized clinical trial so far. Our aim was to investigate whether isoflurane is cardioprotective in an experimental SIC model.

Methods: We induced SIC-like cardiac dysfunction in rats with intraperitoneal injection of isoprenaline (50 mg/kg) and performed this study in two parts. First, we pre-treated rats with isoflurane (1.5%, n = 12), pentobarbital (50 mg/kg, n = 12) and ketamine (80 mg/kg, n = 12) and compared to controls (n = 12). We used glyburide, an ATP-dependent potassium channel blocker (n = 6), to test whether isoflurane-protection is mediated through K_{ATPm}. In a second set of experiments, we treated rats with two different doses of isoflurane i.e. 0.75% (n = 12) and 1.5% (n = 12) before induction of SIC and compared to controls. We assessed left ventricular function and morphology in all rats by transthoracic echocardiography. We also measured peak body temperature, blood gases, acid–base homeostasis, blood pressure and heart rate.

Results: The extent of apical akinesia was lowest and cardiac function was best in the isoflurane treated rats. The protective effects were not attenuated by glibenclamide. Higher dose of isoflurane was more cardioprotective than the lower dose. This was persistent after the adjustment for changes in hemodynamics and blood biochemistry induced by anesthesia.

Conclusions: Isoflurane prevented SIC-like cardiac dysfunction in rats. This protection was not mediated via K_{ATPm}. Our study provides an experimental foundation for future clinical trials in SIC.

Translational perspective:

No treatment exist for stress-induced cardiomyopathy (SIC).

We studied the effects of isoflurane anaesthesia in a rat model of SIC.

Isoflurane dose-dependently protected against SIC in two different experimental setups.

Our study provides an experimental foundation for future clinical trials on isoflurane treatment in SIC.

1. Introduction

Stress-induced cardiomyopathy (SIC), or takotsubo cardiomyopathy, is a novel syndrome with a clinical presentation that is very similar to acute myocardial infarction. SIC is trigged by emotional or physical stress and is characterized by rapid development of extensive but reversible left ventricular apical akinesia that cannot be explained by coronary artery obstruction. Patients with SIC may develop lethal complications including malignant arrhythmias, cardiogenic shock, and ventricular rupture [1] and mortality is similar to acute myocardial infarction. SIC is common in patients hospitalized in intensive care units [2].

The optimal treatment for SIC is not established and many of these patients are treated with medications used for myocardial infarction and heart failure. However, this treatment extrapolation may have catastrophic consequences [3]. No therapeutic intervention for SIC has been evaluated in randomized clinical trial so far. The absence of randomized trials may be a consequence of our limited knowledge about the mechanisms behind SIC and therefore difficulty to define strategies for appropriate pharmacological interventions. For this reason, relevant animal models are valuable for studies about mechanisms involved in SIC as well as for preclinical evaluation of promising therapeutic interventions for future clinical studies.

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We have recently established an experimental SIC model in the rat that reproduces the most important clinical phenomena of SIC in humans including hypercontractility of basal segments [4]. Our findings have been recently reproduced by others [5]. During our work with development of the model and optimization of anesthesia we observed that isoflurane protected against isoprenaline-induced SIC-like cardiac dysfunction. Consequently, we designed this study to test the hypothesis that isoflurane attenuates or prevents SIC-like cardiac dysfunction.

2. Methods

2.1. Animals

All animal work was performed in accordance with the NIH guidelines for the use of experimental animals and the study protocol was approved by the Animal Ethics Committee at the University of Gothenburg. We used 112 10 week old Sprague–Dawley rats. The rats were housed in a temperature-controlled (25 °C) facility with a 12-h light/dark cycle and had free access to food and water. The study protocol consisted of two parts (Fig. 1). In the first part, we tested in 54 rats whether isoflurane is more protective than pentobarbital, and ketamine and whether isoflurane's protective effect can be blocked with glyburide. In the second part, we investigated in 36 rats whether the protective effects of isoflurane are dose-dependent.

2.2. Rat model of stress-induced cardiomyopathy

To test the hypothesis that isoflurane may be protective in SIC we used SIC-like model in rats that was previously described by our group. We induced SIC-like condition by injecting a bolus dose (50 mg/kg) of isoprenaline intraperitoneally. This dose causes left ventricular (LV) apical akinesia (apical ballooning) and characteristic hypercontractility of the basal LV segments similar as in humans in approximately 70% of rats [5] (Fig. 2 and Supplemental video).

2.3. Evaluation of cardiac function

We used high-resolution transthoracic echocardiography for non-invasive evaluation of left ventricular function and morphology. Our equipment for echocardiography was VisualSonics 770 VEVO imaging station which includes an integrated rail system for steady positioning of the ultrasound probe. To optimize the acoustic window we removed fur from the chest with an electrical clipper and hair-removing gel. We used a 35 MHz linear transducer (RMV 707) to obtain an optimal parasternal long axis cine loop, defined as an image that provides clearly visible mitral and aortic valves and maximum distance between the aortic valve and the cardiac apex. All cine loops consisted of >1000 frames/s and were acquired using ECG-gated kilohertz visualization technique. The extent of akinesia was traced in the long axis and expressed as percentage of total LV endocardial length. LV apical ballooning was defined as LV akinesia extending >20% of the LV endocardial length. End-diastolic and end-systolic LV volumes were estimated in the parasternal long axis projection by the prolate-ellipsoid formula [6], volume $= 8A^2 / 3\pi L$, where A is the LV area and L is the LV length. Ejection fraction was estimated as EF = (EDV − ESV) / EDV and stroke volume (SV) as SV = EDV − ESV, where EDV is the end-diastolic volume and ESV is the end-systolic volume. Cardiac output (CO) was calculated as CO = SV × heart rate. All echocardiographic analyses were performed by an echocardiographer who was blinded to the treatment.

2.4. Evaluation of isoflurane in experimental SIC

2.4.1. Comparison between isoflurane, pentobarbital and ketamine

We randomized rats into four groups: pentobarbital (50 mg/kg, n = 12), ketamine (80 mg/kg, n = 12), isoflurane (1.5% inhaled concentration n = 12) and controls (n = 12). The randomization was performed 15 min before injection of isoprenaline (Fig. 1). To test whether the protective effect of isoflurane is mediated through K_{ATPm}-channels we included one additional group (n = 6) which was pretreated with glyburide (1 mg/kg), a K_{ATPm}-channel blocker. Pentobarbital, ketamine and glyburide were injected intraperitoneally 15 min before administration of isoprenaline. The rats were breathing spontaneously through a custom-made mask. Rats randomized to isoflurane inhaled a mixture of isoflurane (1.5%) and air (1.5 l/min). Stable body temperature was maintained with a heating pad set to 36 °C. We used transthoracic echocardiography to analyze cardiac function and morphology 90 min after injection of isoprenaline. Control rats were awake for 80 min post isoprenaline but were anesthetized with isoflurane immediately before echocardiography.

2.4.2. Dose-dependent effect of isoflurane

We designed this experiment to test whether isoflurane's protective effects are dose-dependent. To facilitate administration of isoflurane we used mechanical ventilation. All rats were first anesthetized with intraperitoneal ketamine (100–125 mg/kg) and (midazolam 10–12.5 mg/kg) and placed on a heating pad set to 38 °C. We inserted a plastic cannula BD Venflon Pro; 0.9 or 1.1 mm (Becton, Dickinson and Company) into the right carotid artery and injected 75 IU of heparin to avoid catheter clotting. The catheter was then connected to a transducer attached to the Biopac 100A acquisition system for continuous recordings of arterial blood pressure. Heart rate was calculated from the pulse pressure.

After tracheotomy, a tube was inserted into the trachea and connected to the small-animal ventilator (Harvard Rodent Ventilator model 683) for assisted respiration. We started ventilation with tidal volumes based on body weight, i.e. 2.5 ml (<300 g), 3.0 ml (300–350 g) or 3.5 ml (>350 g). Respiratory rate was set to 80 breaths/min. If the rats breathed spontaneously, we increased stepwise both respiratory rate (by 5 breaths/min) and tidal volume (by 0.5 ml to a maximum of 4.5 ml) until spontaneous breathing ceased. In a pilot study (n = 10), we found that this protocol resulted in the most stable pCO_2 values (4.5–5.5 kPa). Arterial blood pressure and heart rate were recorded continuously while body temperature was recorded every 10 min. Sixty minutes after bolus of isoprenaline we performed second arterial blood gas analysis.

Once adequate baseline ventilation had been achieved, we randomized rats to receive either 0.75% isoflurane (n = 12), 1.5% isoflurane (n = 12) or no isoflurane (n = 12). Arterial blood gas analysis (0.3 ml) was performed 10 min after randomization. We replaced blood volume lost due to the blood sampling with saline. Evaluation of cardiac function was performed 90 min after administration of isoprenaline in all rats. We sacrificed the rats at the end of the experiment with a bolus dose of pentobarbital (50 mg).

2.5. Statistical analysis

Continuous variables are presented as mean ± standard deviation. Normal distribution was assessed by inspecting the distribution of values on a histogram and by the Shapiro–Wilk's test. We used ANOVA (analysis of variance) followed by Fisher's protected least

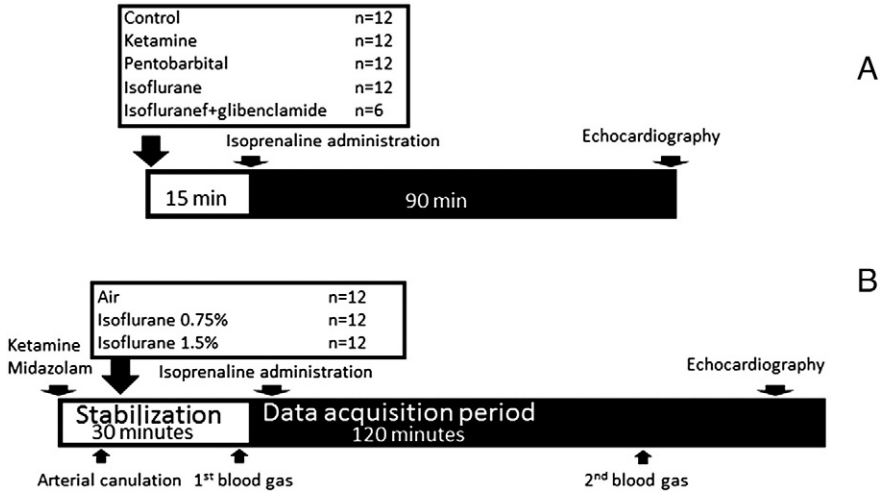

Fig. 1. Experimental setup. A. Evaluation of isoflurane, pentobarbital and ketamine in spontaneously breathing animals. B. Evaluation of dose-dependent effects of isoflurane in mechanically ventilated animals.