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Experimental paper

Heart rate reduction with ivabradine increases ischaemia-induced ventricular fibrillation threshold: Role of myocyte structure and myocardial perfusion☆

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ABSTRACT

Aims: We showed previously that ivabradine (IVA), a selective inhibitor of the cardiac pacemaker I_f current, achieved protection against ischaemia-induced ventricular fibrillation (VF) in pigs by increasing the VF threshold (VFT). This was correlated to the heart rate reduction (HRR), the limitation of monophasic action potential shortening and the reduction of the hypoxic area. This study investigated myocyte ultrastructure and regional myocardial blood flow (RMBF), potentially involved in these cardioprotective effects of IVA.

Methods and results: Myocardial ischaemia was induced in pigs by total 1-min occlusion of the left anterior descending coronary artery following *i.v.* administration of saline ($n = 6$) or IVA (0.25 mg/kg, $n = 6$). Electrophysiological and haemodynamic parameters, the hypoxic area and the presence of myocyte ultrastructural lesions were evaluated. The RMBF was assessed using positron emission tomography following ischaemia/reperfusion in IVA (0.25 mg/kg, *i.v.*, $n = 6$) or vagal stimulation ($n = 4$) groups. Compared with saline, IVA induced a 32% HRR ($p < 0.01$), a 2.9-fold increase in the VFT ($p < 0.001$) and a reduction of the hypoxic area without any change in left ventricular dP/dt_{max}. IVA preserved cardiomyocyte morphology, particularly mitochondrial ultrastructure. Compared with baseline, RMBF during reperfusion was increased in the hypoxic area following IVA administration (+218% *vs.* +97%, $p < 0.05$) or vagal stimulation (+195% *vs.* +127%, $p < 0.05$). This increase was sharply reduced by atrial pacing in IVA-group.

Conclusion: IVA exerts a cardioprotection from ischaemia-induced VF by increasing RMBF and preserving cardiomyocyte and mitochondrial ultrastructure, which opens new perspectives regarding potential targets that would be involved in the anti-ischaemic effects of IVA.

1. Introduction

Ventricular fibrillation (VF) is a well-known complication of acute myocardial ischaemia and is a major cause of sudden cardiac death in patients with ischaemic coronary disease.[1,2] Since the antiarrhythmic/antifibrillatory drugs usually used to prevent such episodes of VF did not show any benefit on mortality and were even associated with higher mortality in large, randomised, placebo-controlled trials,[3] there still exists a medical need for new cardioprotective agents. Indeed, the incidence of primary VF during acute myocardial ischaemia is high (estimated between 2 and 19% depending on the definition of primary fibrillation[4]) and results in significant mortality. We previously demonstrated that sinus tachycardia enhances the risk of ischaemia-induced VF.[5–8] Ivabradine (IVA), a selective inhibitor of the I_f pacemaker current resulting in heart rate reduction (HRR), has been approved for the treatment of stable angina.[9–11] Ivabradine could provide a new therapeutic approach in the prevention of sudden death due to VF induced by ischaemia which is often associated with tachycardia. Previously, in a pig model of VF, we reported that HRR induced by acute administration of IVA provide dose-dependent protection against VF induced by a transient (1 min) total ligation of the left anterior descending (LAD) coronary artery.[12] However, the exact

Abbreviations: dMAP, duration of monophasic action potential; HR, heart rate; HRR, heart rate reduction; IVA, ivabradine; LAD, left anterior descending; LV, left ventricule; LV-d*P*/dtmax, maximum first derivative of LV pressure; mBP, mean blood pressure; PET, positrons emission tomography; RMBF, regional myocardial blood flow; ROI, region of interest; SDH, succinodeshydrogenase; VF, ventricular fibrillation; VFT, ventricular fibrillation threshold.

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mechanisms underlying this cardioprotective effect remain to be elucidated. In models of prolonged myocardial ischaemia, ultrastructural lesions were reported at the cellular level, particularly on mitochondrial shape, which could lead to electromechanical dysfunction.[13] Moreover, an increase in HR has been associated with mitochondrial injuries in a similar experimental setting.[14] So, our hypothesis is that HRR induced by IVA could protect heart against injuries due to ischaemia and tachycardia, which conduct to VF. Also, Heusch et al.[15] showed that IVA-induced HRR was associated with a reduction of the infarct size when administered before, during or at the end of a 90-min controlled coronary artery hypoperfusion and 120-min reperfusion, suggesting that treatment with IVA could preserve the myocardium from ischaemia-induced ultrastructural lesions.

The objective of our study was thus to further investigate the consequences of transient but total coronary occlusion at the morphological and functional levels and to evaluate the potential benefits of HRR induced by IVA on: (1) the electrical threshold of VF (VFT); (2) the prevention of structural lesions in cells and mitochondria and (3) on regional myocardial blood flow (RMBF) determined using positron-emission tomography (PET) as a non-invasive procedure.

2. Materials and methods

The present study design was approved by the animal care committee of Claude Bernard University (Lyon, France).

Protocol 1: this protocol studies the impact of IVA on myocardial morphological and functional changes.

2.1. Animal preparation

The experiments were conducted on a total of 12 domestic male pigs (Landrace strain) in order to be as comparable as possible of with human anatomy coronary system, weighing 20–25 kg (3 months). The animals were premedicated with 20 mg/kg ketamine (Roche Neuilly-sur-Seine cedex, France) intramuscularly 30 min before the experiment. Anaesthesia was induced by an *i.v.* bolus of 3 mg/kg propofol (via the marginal ear vein) and maintained with an *i.v.* perfusion of 100 mg/kg alpha-chloralose (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) injected via jugular vein. Alpha-chloralose was has been used because as it presents of its minimal depression effect on autonomic reflex activity, particularly on the heart rate.[16,17] Parameters of ventilation have been previously reported.[12] Pigs were each placed on the back and a wide thoracotomy was performed. The heart was exposed and the pericardium opened. An ECG was recorded in the standard limb leads on a Mingograf 34 electrocardiograph.

2.2. Induction of ischaemia

The LAD coronary artery was dissected free near its origin and a snare looped around it in preparation for occlusion. Ischaemia was induced by complete and brief (1-min) occlusion of the proximal LAD by tightening the snare around the artery. Repeated abrupt coronary occlusions were performed at 15-min intervals (Fig. 1A). Ischaemia was evaluated by the cyanotic appearance of the unperfused zone distal to the occlusion and by the ST-segment shift in the lead II on the ECG.

2.3. Treatments

6 pigs received the dose of 0.25 mg/kg IVA (Institut de Recherches Internationales Servier, Courbevoie, France) administered as a slow *i.v.* bolus (1 min) through the jugular vein, and 6 pigs received the same volume of saline (control group) in similar conditions (Fig. 1A). The dose of 0.25 mg/kg of IVA was chosen because it was previously demonstrated as being the lowest effective dose able to increase the VFT.[12]

2.4. Electrophysiological recordings

Heart rate (HR) was monitored on the ECG in lead II and recorded at the end of the 1-min coronary occlusion, just before VFT determination.

VF was triggered at 1-min coronary occlusion by the application of a ventricular pacing in the hypoxic area as described previously,[12] to evaluate the VFT. Two baseline and four post-injection VFT determinations were performed alternately under ventricular pacing at "spontaneous HR" (*i.e.* HR recorded just before induction of ischaemia) and at a fixed rate of 300 ms (~200 bpm). Pacing at 300 ms was performed in order to study the impact of HRR *per se* by counteracting the HR-lowering effect of IVA (Fig. 1A). A 360 J shock applied to the thoracic wall was used for defibrillation (D802 defibrillator, Siemens, Erlangen, Germany).

Myocardial electrical activity was analysed by the measure of duration of monophasic action potential (dMAP) in the centre of hypoxic area as described previously.[12]

2.5. Haemodynamic parameters

An arterial pressure line was established through a catheter inserted into the left carotid artery and connected to a polygraph (M1166 A, model 66 S, Hewlett Packard Inc., USA) to monitor mean arterial blood pressure (mBP). The increase in LV-dP/dt_{max} was electronically derived using Acknowledge software (Biopac System Inc., Santa Barbara, CA, USA) from intraventricular pressure signals obtained through a catheter positioned in the LV from the right carotid artery. All haemodynamic parameters were measured 1 min before and after each coronary artery occlusion, before and after IVA administration.

2.6. Morphological and functional changes

Immediately after euthanasia, all of the animals' hearts were excised for the determination of succinodeshydrogenase (SDH) activity to determine the extent of the hypoxic area as described in our previous study.[12] Samples of the LV were taken in the centre of the hypoxic area analysis of cardiomyocyte ultrastructure using transmission electron microscopy. They were fixed in 4% glutaraldehyde – 0.3 M Na cacodylate–HCl (pH 7.4; 2 h), post-fixed with 2% osmium tetroxide – 0.15 M Na cacodylate–HCl (pH 7.4; 1 h), dehydrated with ethanol and embedded in Epon (60 °C; 72 h). Then, 60–90 nm-thick sections were cut using an ultramicrotome (RMC/MTX; Elexience), contrasted with uranyl acetate and lead citrate. Sections were observed using a JEOL 1200CX transmission electron microscope to evaluate the number of nuclei with disrupted membranes and irregular chromatin agglomeration (among the 20 nuclei), the number of disrupted or swollen sarcolemna (among the 20 sarcolemna), the number of cell junction disruptions (among the 20 cell junctions), the number of necrotic cardiomyocytes (among the 20 cardiomyocytes), the number of displaced, swollen, or degranulated mitochondria and disrupted mitochondrial crests (among the 200 mitochondria) and the number of capillaries with thickening of the basal membrane and abnormal chromatin distribution in the nuclei (among the 20 capillaries).

Protocol 2: this protocol aimed at testing the impact of IVA on RMBF in our pig model of experimental myocardial ischaemia/reperfusion.

RMBF was first determined in 10 pigs 15 min before, just after and 15 min after induction of myocardial ischaemia induced by a