ORIGINAL ARTICLE

Mimicking Preconditioning by Vagal Stimulation. Effects on Ventricular Function in a Chronic Experimental Model

Mimetización del precondicionamiento por activación vagal. Efectos sobre la función ventricular en un modelo experimental crónico

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ABSTRACT

Background: Previous studies have shown that preischemic vagal electrostimulation (pVS) reduces acute myocardial infarct size, without a significant improvement on ventricular function within the two-hour reperfusion period. It is unknown which are the long-term effects of pVS on left ventricular function (LVF).
Objectives: The aim of this study was to analyze whether the protective effects of brief pVS on acute infarct size improves LVF in a chronic myocardial ischemia-reperfusion model.
Methods: FVB mice were subjected to 45-minute regional myocardial ischemia followed by 2 hours of reperfusion or 28-day post-reperfusion follow-up with or without 10-minute pVS. Infarct size (IS) was measured with 2,3,5-triphenyltetrazolium chloride, and LVF was assessed by echocardiography and left ventricular catheterization.
Results: Preischemic vagal stimulation reduced IS from 66.8±3.2% to 43.2±1.6% (p <0.001) at 2 hours of reperfusion, without a favorable LVF response. At 28 days, the pVS group exhibited LVF improvement, with lower left ventricular end-diastolic pressure (4.44±1 vs. 6.91±1 mmHg in the control group; p<0.05), higher ejection fraction (69.7±2.8% vs. 59.3±3.2; p<0.05), greater shortening fraction (33.4±2.23 vs. 25.8±1.8%; p<0.05) and lower isovolumic relaxation time (25±0.8 ms vs. 30.3±1.2 ms; p<0.05)
Conclusions: In a mice model of myocardial ischemia and reperfusion, mimicking ischemic preconditioning by VS improves the chronic outcome of infarction, resulting in greater LVF recovery.

Key words: Myocardial Infarction - Vagus Nerve Stimulation- Ischemia - Ventricular Remodeling

RESUMEN

Introducción: En trabajos previos demostramos que la electroestimulación vagal preisquémica (EVp) es capaz de reducir el tamaño del infarto agudo de miocardio, sin una mejoría significativa sobre la función ventricular dentro de las dos horas de reperfusión. Se desconocen los efectos de esta modalidad de EV sobre la función ventricular izquierda (FVI) a largo plazo.
Objetivos: Estudiar si los efectos protectores de la EVp breve sobre el tamaño del infarto agudo repercuten en una mejoría de la FVI en un modelo crónico de isquemia y reperfusión miocárdica.
Material y métodos: En ratones FVB se realizó una isquemia miocárdica regional de 45 minutos con 2 horas o 28 días de seguimiento posreperfusión, con o sin 10 minutos de EV preisquémica. Se midió el tamaño del infarto (TI) con cloruro de 2,3,5-trifeniltetrazolio. Se evaluó la FVI mediante ecocardiografía y cateterismo del VI.
Resultados: La EVp redujo el TI medido a las 2 horas de reperfusión de 66,8±3,2% a 43,2±1,6% (p<0,001), sin una respuesta favorable sobre la FVI. A los 28 días, en el grupo con EVp se observó una mejoría en la FVI, evidenciada por una menor presión de fin de diástole del ventrículo izquierdo (4,44±1 vs. 6,91±1 mmHg del grupo control; p<0,05), mayor fracción de eyección (69,7±2,8% vs. 59±3,2%; p<0,05), mayor fracción de acortamiento (33,4±2,23% vs. 25,8±1,8%; p<0,05) y menor tiempo de relajación isovolúmica (25±0,8 mseg vs. 30,3±1,2 mseg; p<0,05).
Conclusiones: En un modelo de isquemia y reperfusión miocárdica en ratones, la mimetización del precondicionamiento isquémico por EV mejora la evolución crónica del infarto y redunda en una mayor recuperación de la FVI.

Palabras clave: Infarto del Miocardio - Estimulación del Nervio Vago - Isquemia- Remodelación Ventricular

Abbreviations

AR	Area at risk	**LVEDP**	Left ventricular end diastolic pressure
EF	Ejection fraction	**LVSP**	Left ventricule sistolic pressure
IR	Ischemia-reperfusion	**pVS**	Preischemic vagal stimulation
IS	Infarct size	**SF**	Shortening fraction
IVRT	Isovolumic relaxation time	**VS**	Vagal stimulation
LVF	Left ventricular function		

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INTRODUCTION

Ischemic heart disease is one of the leading causes of morbidity and mortality worldwide (1) and, according to current predictions, its impact will remain at very high levels beyond 2020. (2) The outcome and prognosis faced by a patient with an ischemic injury is determined in the first place by the extent of the region affected by ischemia, i.e. infarct size (IS). (3) Another important factor is the imbalance of the autonomic nervous system, characterized by increased sympathetic tone and reduced parasympathetic tone.

Undoubtedly, the most effective way of limiting the extent of damage is early reperfusion of the occluded coronary artery, before irreversible ischemic myocardial injury occurs. Despite great improvements have been made in this sense, a significant number of patients progress to adverse left ventricular remodeling and heart failure. Although reperfusion is essential to save the myocardium from ischemic cell death, the sudden input of blood to the hypoxic tissue generates additional damage, known as reperfusion injury. Therefore, in the last years there has been growing interest in the use of myocardial protection techniques, as preconditioning or postconditioning, which reduce ischemia/reperfusion injury. (4)

Dysautonomia is present in cardiovascular diseases and is an independent factor of bad prognosis in patients with ischemic heart disease. Use of β blocking agents to counteract sympathetic hyperactivity is an effective, albeit still insufficient, therapeutic option. (5) On the other hand, the parasympathetic component of dysautonomia has been less studied and favorable therapeutic effects are still lacking. In this sense, clinical studies performed during the last years have demonstrated that electric stimulation of the vagal nerves has beneficial effects on ischemic heart disease (6-9). We have recently shown that brief, continuous vagal stimulation (VS) applied before myocardial ischemia (pVS) or at the onset of reperfusion (rVS) reduces IS, mimicking preconditioning by activating the Akt/GSK-3 muscarinic pathway, and ischemic postconditioning through activation of nicotinic α7 receptors and the JAK2 protein pathway. (10) However, it is unknown if preconditioning by VS has protective effects on long-term ventricular remodeling after early reperfusion. Thus, the aim of this study was to analyze whether the protective effects of brief pVS on acute IS have an impact on LVF improvement in a chronic myocardial ischemia-reperfusion (IR) model.

METHODS

Experimental model. Male FVB mice were anesthetized with an intraperitoneal induction dose of 0.3 mg/kg Avertin (2,2,2-tribromide metanol) (11) and maintenance doses as required, controlling superficial reflexes. Regional myocardial ischemia elicited by 45-minute occlusion of the anterior descending artery was followed by a 2-hour or 28-day reperfusion period. In the chronic experiments, the animals were recovered from anesthesia after wound closure by anatomic planes, receiving tramadol 50 μg/g as analgesic and 50 μg/g cefazolin sodium as antibiotic treatment. Once awake, mice were kept in individual cages until the end of the experimental protocol.

Experimental protocols (Figure 1). After 10 minutes of pVS and 5 minutes recovery without VS, mice were subjected to 45-minute myocardial ischemia followed by reperfusion of the occluded artery, either for 2 hours (pVS+IR-2h group) or 28 days (pVS+IR-28d), and were compared with their respective controls without VS (IR-2h and IR-28d groups) and with corresponding groups with simulated coronary occlusion (Sham groups).

Vagal stimulation. In the animals receiving pVS a bipolar electrode (MLA270 Stimulation Cable, AD Instruments) connected to a neurostimulator (Grass S44 Stimulator) was placed at the cervical level of the right vagus nerve. A constant 10-minute stimulation with rectangular 0.1 ms pulses was performed before ischemia at a frequency of 10 Hz and variable intensity to approximately reduce heart rate prior to stimulation by 10-15%. (12)

Infarct size assessment. After the 2-hour reperfusion period, the acute protocol animals were sacrificed with an overdose of xylazine and ketamine. The coronary artery was ligated again and the ascending aorta was cannulated and perfused with Evans blue solution to measure the area at risk (AR) (not dyed area). The AR was expressed as a percentage of? total left ventricular wall area. The heart was then cut into transverse sections and these were incubated in 1% 2,3,5-triphenyltetrazolium chloride (TTC) solution (12) during 20 minutes to measure IS, which was expressed as a percentage of? left ventricular AR. Digital images were obtained and analyzed with Image Analyzer software (Image-Pro Plus 6.0).

Hemodynamic measurements. Heart rate (HR), left ventricular systolic pressure (LVSP), +dP/dtmax, -dP/dtmax and left ventricular end-diastolic pressure (LVEDP) were recorded throughout the acute protocols and at the end of the chronic protocols. To perform these measurements, the right common carotid artery was dissected and a catheter was introduced into the left ventricle associated to a preamplifier and Power Lab system and connected to a computer with LabChart software.

Echocardiographic measurements. At the end of the 28-day follow-up period, the animals were anesthetized with 290 mg/kg of 2.5% Avertin (Sigma-Aldrich) solution to perform an echocardiographic study with an Acuson Sequoia

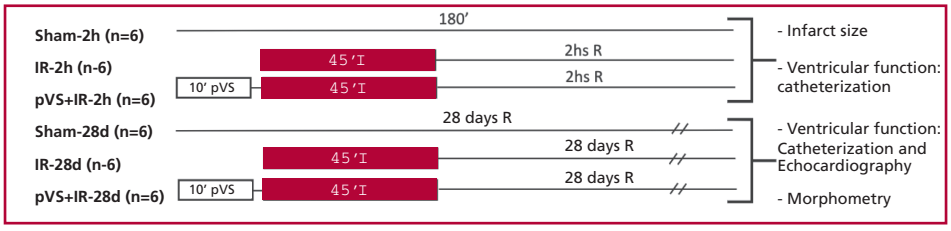

Fig. 1. Schematic representation of experimental protocols. pVS: Preischemic vagal stimulation. IR: Ischemia-reperfusion. d: days. h: hours.

C512 ultrasound system and a linear 14 MHz probe. Ejection fraction (EF) and shortening fraction (SF) were calculated to assess systolic function, and diastolic function was evaluated from the isovolumic relaxation time (IVRT).

Statistical analysis

Results were expressed as arithmetic mean and standard error. Hemodynamic variables were analyzed using ANOVA for repeated measures followed by the Bonferroni test. The AR and IS were analyzed with Student's t test. A p value <0.05 was considered significant.

Ethical considerations

The experimental model of this study conformed to the Animal Care Committee of the School of Medicine of Universidad de Buenos Aires (Res CD N° 339/18).

RESULTS

Effects of vagal stimulation on infarct size

Figure 2A shows the AR of the groups at 2-hour reperfusion without and with pVS (IR-2h: 50±2.75%; pVS+IR-2h: 51.9±3.19%, p=NS); as expected, no significant differences were found between groups. Preischemic vagal stimulation reduced IS compared with the control group (43.2±1.6% vs. 66.8±3.2%, respectively, p<0.001) (Figure 2B).

Effects of vagal stimulation on ventricular function and remodeling

Table 1 shows no baseline differences in HR, LVSP, LVEDP, +dP/dtmax and –dP/dtmax between acute protocols.

During ischemia, LVEDP increased significantly both in the IR-2h group (14.1±3 mmHg) as in the pVS+IR-2h group (11.23±2 mmHg) (p<0.05) and these values tended to improve during reperfusion. There was also a reduction of LVSP, +dP/dtmax and –dP/dtmax. Preischemic vagal stimulation reduced HR by 16.5% with respect to the baseline value before stimulation, but did not favor a better recovery of LVF compared with the control group.

Table 2 shows hemodynamic values obtained by left ventricular catheterization at the 28-day post-reperfusion follow-up. No significant differences were observed in HR, LVSP, +dP/dtmax and –dP/dtmax. However, a significant increase of LVEDP was found in the IR-28d group (6.91±1 mmHg) compared with the sham-28d group (3.81±0.2 mmHg) (p<0.01). Interestingly, preischemic VS significantly improved LVEDP reducing it to 4.47±1 mmHg (p<0.05).

Figure 3 illustrates EF (A), SF (B) and IVRT (C) results evaluated at the 28-day post-reperfusion follow-up. Compared with the sham group, the IR-28d group evidenced a decrease in EF (74±2% vs. 59±3%, p<0.05) and SF (37±2% vs. 26±2%, p<0.05) and an increase in IVRT (19±1 ms vs. 30±1 ms, p<0.05). These variables improved with pVS (EF: 70±3%, SF: 33±2% and IVRT: 25±1 ms, p<0.05 vs. IR-28d).

Figure 4 shows that VS did not reduce left ventricular weight compared with the control group.

DISCUSSION

In the present work we show that 10-minute electric stimulation of the right vagal nerve before ischemia reduces IS measured after a 2-hour reperfusion period in an IR mice model. Interestingly, mimicking preconditioning by VS improves the chronic outcome of ventricular function assessed by left ventricular echocardiography and catheterization.

Previous studies have shown that VS or the acetylcholine neurotransmitter can have cardioprotective effects. Effectively, it is known that in in vitro models with exogenous acetylcholine administration, IS is reduced by preconditioning activation. (13) Moreover, Cavillo et al. in a rat model of 30-minute ischemia followed by reperfusion, demonstrate that prolonged VS during ischemia markedly reduces IS versus the control group, attributing this finding to a significant reduction in the amount of macrophages and polymorphonuclear leukocytes infiltrating the myocardium. This reduction of inflammation was also evidenced by decreased levels of circulating proinflammatory cytokines.

We have shown in previous studies that pVS reduces IS both in rabbits and mice. We thus demonstrated for the first time that the increase of acute parasympathetic activity can mimic classic preconditioning through muscarinic receptors, activating the Akt/GSK-3 pathway. (10) Surprisingly, this IS reduction did not reflect on LVF protection assessed 2 hours after reperfusion, in agreement with the present results where no improvement was found in LVSP, +dP/dtmax and –dP/dtmax. It is possible that the lack of left ventricular recovery could be attributed to the still stunned viable myocardial after reperfusion, needing more than 2 hours to achieve functional recovery. In

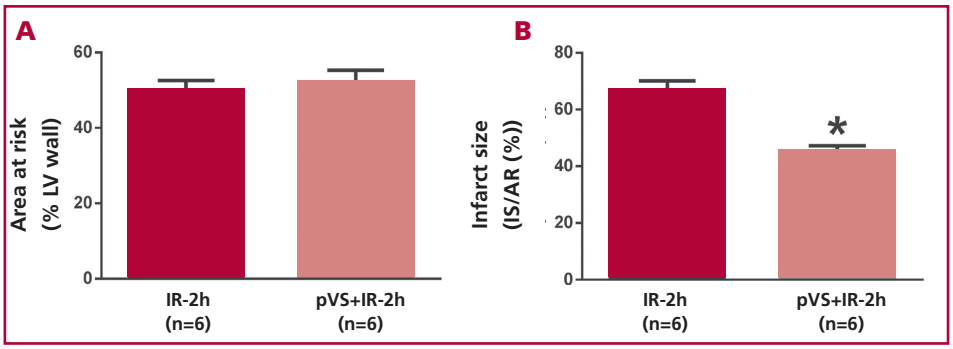

Fig. 2. Area at risk (**A**) and infarct size (**B**) of groups undergoing a 2-hour reperfusion period. LV: Left ventricular; IR: Ischemia-reperfusion; pVS: Preischemic vagal stimulation; IS: Infarct size; AR: Area at risk (*p<0.001 vs. IR-2h)

Table 1. Ventricular function and heart rate of groups with 2-hour post-reperfusion follow-up

	GROUPS	Baseline	5 min of VS	PreIsch. control	45 min Isch.	5 min Rep.	60 min Rep.	120 min Rep.
HR	Sham- 2h	488±26	486±25	515±43	490±29	489±29	498±20	490±22
(bpm)	IR-2h	453±7	446±8	473±29	477±8	486±11	485±6	499±10
	pVS+IR-2h	479±18	402±25	522±17	509±9	507±13	478±32	456±35
LVSP	Sham-2h	100±7	100±7	103±3	98±2	97±5	99±2	96±2
(mmHg)	IR-2h	95±3	94±4	93±2	79±3 *	78±3 *	79±3 *	79±3 *
	pVS+IR-2h	92±5	92±6	91±5	80±6	79±5	80±5	77±5
LVEDP	Sham-2h	2.78±0.1	2.91±0.2	3.49±0.2	3.25±0.2	3.2±0.3	2.91±0.,3	3±0.4
(mmHg)	IR-2h	2.93±0.2	3.45±0.2	3.09±0.2	14.1±3 *	9.36±2	6.14±1 †	4.59±0.5 †
	pVS+IR-2h	3.51±0.3	3.77±0.3	3.64±1	11.23±2 *	5.76±0.5 †	5±1 †	4.19±0.4 †
+dP/dtmax	Sham-2h	8,843±952	8,769±874	9,745±100	8,330±238	8,243±557	8,566±416	7,642±425
(mmHg/s)	IR-2h	7,163±594	6,817±674	6,962±427	5,497±373	5,133±408	5,192±393	5,108±386
	pVS+IR-2h	6,306±1061	6,002±1226	7,379±1,439	5,359±760	5,042±789	5,216±753	4,600±673
-dP/dtmax	Sham-2h	-6,563±511	-6,337±493	-6,497±64	-6,986±95	-6,917±301	-7,337±203	-6,892±188
(mmHg/s)	IR-2h	-6,506±330	-6,509±456	5,923±216	-4,659±295*	-4,842±309 *	-4,887±345 *	-4,743±349 *
	pVS+IR-2h	-6,162±914	-5,817±916	-5,718±611	-4,516±638	-4,599±599	-4,689±573	-4,114±571

IR: Ischemia-reperfusion; pVS: Preischemic vagal stimulation; Isch: Ischemia; Rep: Reperfusion; min: Minutes. HR: Heart rate; LVSP: Left ventricular systolic pressure; LVEDP: Left ventricular end-diastolic pressure. +dP/dtmax: maximum rate of pressure rise of left ventricular pressure; -dP/dtmax: maximum rate of pressure fall of left ventricular pressure. bpm: beats per minute. (*p<0,05 vs. basal y Sham-2h; † p<0,05 vs. 45 min Isq., Sham-2h)

Table 2. Ventricular function and heart rate of groups with 28-day post-reperfusion follow-up

GROUPS	HR (bpm)	LVSP (mmHg)	LVEDP (mmHg)	+dP/dtmax (mmHg/s)	-dP/dtmax (mmHg/s)
Sham-28d	461±11	95±5	3.81±0.2	7,348±1,065	-6,960±530
IR-28d	465±12	99±1	6.91±1 *	8,438±226	-7,289±283
pVS+IR-28d	494±15	96±5	4.47±1†	7,922±868	-7,204±281

IR: Ischemia-reperfusion. pVS: Preischemic vagal stimulation; HR: Heart rate; LVSP: Left ventricular systolic pressure; LVEDP: Left ventricular end-diastolic pressure; +dP/dtmax: maximum rate of pressure rise of left ventricular pressure; -dP/dtmax: maximum rate of pressure fall of left ventricular pressure. (*p<0.01 vs. Sham-28d and †p<0.05 vs. IR-28d).

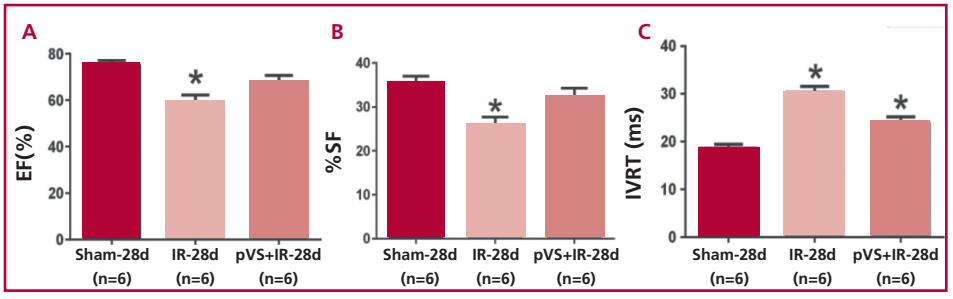

Fig. 3. Percent ejection fraction (%EF) (**A**), shortening fraction (%SF) (**B**) and isovolumic relaxation time (IVRT) (**C**) in groups with 28-day reperfusion. IR: Ischemia-reperfusion; pVS: Preischemic vagal stimulation. d: days (*p <0.05 vs. sham-28d).

consonance with this hypothesis, we observed that at 28 days post-reperfusion, there was an improvement in LVF assessed both by echocardiography and catheterization. However, it is important to point out that pVS protection on late LVF could not only be due to functional recovery of the viable myocardium but also to structural changes as a consequence of the benefits afforded on left ventricular remodeling.

Conversely, little is known whether the protective effects of pVS provide long term benefits on left ventricular remodeling and function. Li et al. (14) described that 6-week VS improved the survival of rats with heart failure by preventing the progress of pump dysfunction and cardiac remodeling, evidenced as reduction in left ventricular weight. Another similar study showed in a rabbit model that 3-day stimulation since the onset of ischemia leads to improved ventricular function. (8) Echocardiography indicated that both SF as end-systolic and end-diastolic left ventricular diameters improved in the VS group. However, these studies were performed with prolonged VS, with direct effects on the inflammatory response and other variables that have a direct impact on cardiac chronic ischemic injury.

An interesting finding of our study was the clear improvement of LVF in the pVS group after four

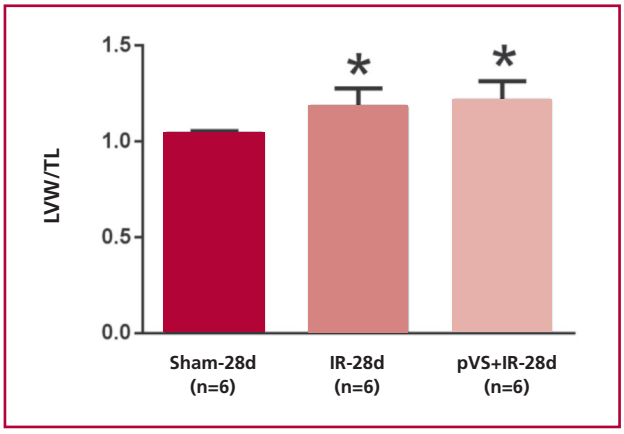

Fig. 4. Left ventricular weight (LVW) corrected by tail length (TL). A significant increase in left ventricular weight was observed in the IR-28d and pVS+IR-28d groups vs. Sham-28d (* p<0.01). IR: Ischemia-reperfusion; pVS: Preischemic vagal stimulation.

weeks of evolution, but that this improvement was not accompanied by reduction in left ventricular weight, suggesting a dissociation between morphological and functional aspects of chronic ventricular remodeling. In this sense Agarwal et al. (15) described an inverse phenomenon. In rats with myocardial ischemia without reperfusion they obtained a reduction in left ventricular remodeling that was not accompanied by an improved functional response. However, in this work the authors performed a prolonged 6-week VS, also suggesting possible differential effects in the long-term response of short or prolonged VS.

Although these are still preliminary results, they support further studies on cardiac remodeling using histological techniques and advance in the analysis of the mechanisms involved in myocardial protection.

CONCLUSIONS

The present study demonstrated for the first time that in a mice model undergoing 45-minute myocardial ischemia followed by reperfusion, brief preischemic vagal stimulation reduces infarct size and improves the long-term outcome of the ischemic heart, evidenced by a better recovery of left ventricular function.

Conflicts of interest

None declared.

(See authors' conflicts of interest forms on the website/ Supplementary material)

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