

Myocardial Infarction in a Murine Model of Cardiac Sympathetic Hyperactivity

Infarto de miocardio en un modelo murino de hiperactividad simpática cardíaca

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ABSTRACT

Background: Dysautonomia is one of the main pathophysiological mechanisms that define the prognosis of ischemic heart disease and heart failure. The search for new treatment opportunities requires a deeper understanding of the cardiac effects of chronic sympathetic activation.

Objective: The aim of this study was to analyze left ventricular infarct size and ventricular function in a transgenic mouse model with overexpression of the cardiac G_s-α protein, in the context of myocardial ischemia/reperfusion and chronic infarction.

Methods: Transgenic mice (TG) overexpressing cardiac G_s-α and wild-type (WT) mice were subjected to 30-minute regional myocardial ischemia followed by 2-hour reperfusion (IR), or non-reperfusion (I) with a 28-day follow-up period. Infarct size (IS) was quantified using 2,3,5-triphenyltetrazolium chloride, and left ventricular function was evaluated by echocardiography and hemodynamic study. Each experimental group was accompanied by a control group (WT/TG Sham-2hrs and WT/TG Sham-28d).

Results: There were no significant differences in IS after IR between TG and WT mice ($57.3 \pm 3.5\%$ vs. $59.2 \pm 2.5\%$, respectively, $p = \text{NS}$). A significant increase in heart rate was evident in TG mice throughout the protocol. A decrease in ejection fraction (WT: Sham-28d: $82 \pm 2.4\%$ vs. I-28d: $44 \pm 4\%$ and TG: Sham-28d $89 \pm 2\%$ vs. I-28d $42 \pm 3\%$; $p < 0.05$) was observed together with a decrease in shortening fraction and left ventricular fractional area changes compared with baseline values and their respective control (Sham) groups. However, no differences were observed between the WT and TG groups.

Conclusions: Cardiac G_s-α protein overexpression does not increase infarct size or modify left ventricular function in acute ischemia/reperfusion and chronic infarction compared with their respective controls.

Key words: Dysautonomia - Ischemia/Reperfusion - Myocardial Infarction - G_s-Alpha Protein

RESUMEN

Introducción: La disautonomía es uno de los mecanismos fisiopatológicos principales que marcan el pronóstico de la cardiopatía isquémica y la insuficiencia cardíaca. La búsqueda de nuevas oportunidades de tratamiento requiere un conocimiento más profundo de los efectos cardíacos de la activación simpática crónica.

Objetivos: Estudiar el tamaño del infarto y la función ventricular izquierda en un modelo de ratones transgénicos con sobreexpresión de la proteína G_s-α cardíaca en el contexto de la isquemia/reperfusión miocárdica y el infarto crónico.

Material y métodos: Ratones transgénicos (TG) con sobreexpresión cardíaca de la subunidad alfa de la proteína G_s y sus respectivos controles wild-type (WT) fueron sometidos a isquemia miocárdica regional de 30 minutos con 2 horas de reperfusión (IR) o un infarto sin reperfusión (I) de 28 días de evolución. Se cuantificó el tamaño del infarto (TI) con cloruro de 2,3,5-trifeniltetrazolio y se evaluó la función ventricular izquierda mediante ecocardiografía y estudio hemodinámico.

Resultados: No hubo diferencias significativas en el TI luego de la IR entre los ratones TG y WT ($57,3 \pm 3,5\%$ vs $59,2 \pm 2,5\%$, respectivamente, $p = \text{NS}$). La frecuencia cardíaca en los ratones TG fue mayor durante todo el protocolo llevado a cabo. Se observó un descenso de la fracción de eyección (WT: Sham-28d: $82 \pm 2,4\%$ vs I-28d: $44 \pm 4\%$ y TG: Sham-28d $89 \pm 2\%$ vs I-28d $42 \pm 3\%$; $p < 0,05$) conjuntamente con una disminución de la fracción de acortamiento (FA), y los cambios del área fraccional (CAF) del ventrículo izquierdo (VI) en comparación con los valores basales y sus respectivos grupos controles. Sin embargo, no se observaron diferencias entre los grupos WT y TG.

Conclusión: la sobreexpresión de la proteína G_s-α cardíaca no aumenta el tamaño del infarto ni modifica la función ventricular izquierda en la isquemia/reperfusión aguda y en el infarto crónico en comparación con sus respectivos controles.

Palabras clave: Disautonomía - Isquemia Reperfusión - Infarto de Miocardio - Proteína G_s-Alfa

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INTRODUCTION

Cardiovascular diseases, especially ischemic heart disease and its long-term consequences, are still the pathologies with the highest number of deaths worldwide. (1) The increase of sympathetic tone and the reduction of parasympathetic tone in dysautonomia found in cardiovascular diseases, worsen their prognosis. (2, 3)

The abrupt or gradual fall of cardiac output in ischemic heart disease initiates neurohumoral compensation mechanisms that aim to sustain blood pressure and the perfusion of noble organs avoiding the development of cardiac failure. (4) However, extended neurohumoral activation, especially of the sympathetic nervous system, generates a sliding curve that worsens heart failure by increasing peripheral vascular resistance and expanding extracellular volume. (5) The ensuing increase in preload and afterload have a negative impact on metabolism and cardiac function. In addition to these systemic pathophysiological mechanisms, direct cardiac sympathetic stimulation aims to increase contractility by activation of the β -adrenergic receptor- G_s -adenylyl cyclase pathway. But prolonged sympathetic stimulation enhances myocardial oxygen demand, that paradoxically could further impair cardiac function. (6)

It is well known that sympathetic activation is a marker of heart failure status and a negative predictive factor of morbidity and mortality. (7) Both β -adrenergic blockade and parasympathetic activation have shown favorable effects on ischemic heart disease and heart failure. (6, 8-11) Nevertheless, the prognosis of a large number of patients with ischemic heart disease is still ominous, generating the need for a deeper understanding of its pathophysiological mechanisms in the search of new therapeutic options.

The aim of this study was to assess infarct size and left ventricular function in a transgenic mouse model overexpressing cardiac G_s - α protein in the context of myocardial ischemia/reperfusion and chronic infarction. These transgenic mice are characterized by greater selective β -adrenergic activation in the heart,

manifested by a higher chronotropic and inotropic response. (12) However, it has not yet been shown whether this specific sympathetic stimulation in the heart impacts on infarct size and ventricular function changes in ischemic heart disease.

METHODS

Experimental design

Male 2-to 4-month-old transgenic (TG) mice, selectively overexpressing the alpha subunit of the stimulating G-guanosine 5'-triphosphate protein (G_s - α) were used, and compared with wild type (WT) mice. (13) For the acute experiments, mice were anesthetized with ketamine and xylazine (35 and 5 mg/kg, respectively). Additional maintenance doses were administered as required. The thorax was accessed through the left fourth intercostal space and regional myocardial ischemia was generated. Following surgical incision closure by anatomical planes, animals with chronic follow-up were recovered from anesthesia in individual cages, receiving a dose of tramadol 50 μ g/g as analgesic and Cefazolin sodium 50 μ g/g as antibiotic. (9, 10)

Experimental protocol (Figure 1)

After a stabilization period, the anterior interventricular artery was occluded in acute experiments. In the acute groups, a 30-minute ischemic period was followed by a 2-hour reperfusion (IR) period (WT IR-2hrs and TG IR-2hrs), while in chronic groups, ischemia was without reperfusion (I), with a 28-day follow-up period (WT I-28d and TG I-28d). Each experimental group with ischemia was accompanied by a control group (WT/TG Sham-2hrs and WT/TG Sham-28d).

Infarct size assessment

After a 2-hour reperfusion period, the animals of the acute protocols were euthanized with an overdose of ketamine and xylazine. The reperfused artery was ligated again, and an Evan's blue solution was infused through the ascending aorta to identify the area at risk (non-dyed area). Subsequently, the left ventricle (LV) was cut in transverse sections and incubated in a 1% 2,3,5-triphenyltetrazolium chloride (TTC) solution (8) during 20 minutes to measure the infarcted area. Area at risk (AR) was calculated from digital images using Image Pro Plus 6.0 software, and expressed as percent total left ventricular wall, and infarct size (IS) was expressed as percent left ventricular risk area. (10)

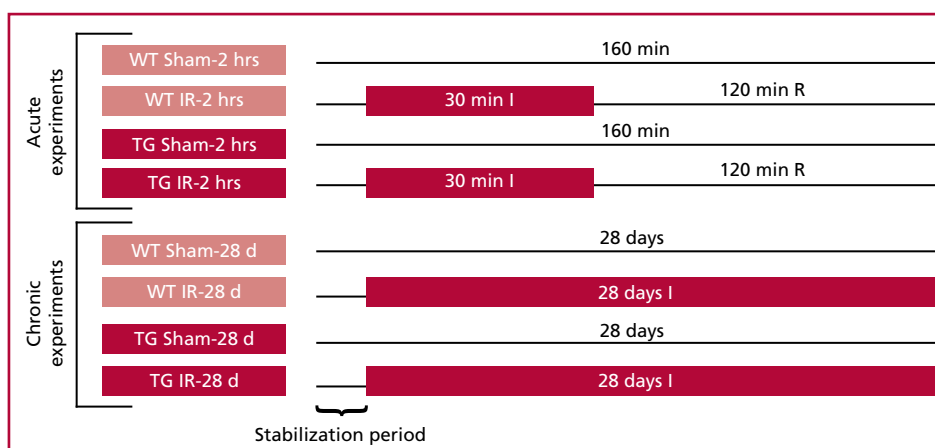


Fig. 1. Diagram representing the experimental design. WT: Wild-type mice; TG: transgenic mice; IR: Ischemia/reperfusion; min: minutes; d: days; hrs: hours; I: Ischemia; R: reperfusion

Hemodynamic measurements

Following right common carotid artery dissection, a catheter was introduced into the LV coupled to a preamplifier and a PowerLab machine connected to a computer with LabChart software. During the entire acute protocol and at the end of chronic protocols, heart rate (HR), left ventricular systolic pressure (LVSP), $+dP/dt_{max}$, $-dP/dt_{max}$ and left ventricular end-diastolic pressure (LVEDP) were recorded. (10)

Echocardiographic measurements

After 28 days of post-ischemic follow-up, mice were anesthetized with 0.3 mg/kg of 2% Avertin (2,2,2-Tribromoethanol) (Sigma Aldrich). (14) to perform echocardiography using an Acuson Sequoia C512 ultrasound machine, with a 14 MHz linear ultrasound transducer. Left ventricular ejection fraction (LVEF), shortening fraction (SF) and fractional area changes (FAC) were calculated to assess systolic function. (9)

Statistical analysis

Results were expressed as mean and standard error of the mean. Analysis of variance for repeated measures followed by Bonferroni post-hoc test was used to analyze hemodynamic variables and Student's *t* test for AR and IS results. A significant difference was considered for $p < 0.05$.

Ethical considerations

The experimental model was approved by the Animal Care Committee of the School of Medicine, University of Buenos Aires (CD Res No. 339/18).

RESULTS

Effects of $G_s\text{-}\alpha$ overexpression on infarct size and ventricular function in acute experiments

Figure 2, panel A shows the AR of WT and TG groups with 2-hour post-ischemic reperfusion period. As expected, no significant differences were found (WT IR-2hrs $43 \pm 2.5\%$; TG IR-2h: $41.9 \pm 3\%$. $p = \text{NS}$) allowing the subsequent infarct size comparisons. Overexpression of protein G_s alpha subunit did not significantly modify the measured IS after 30-minute ischemia and 2-hour reperfusion (WT IR-2hrs $59.2 \pm 2.5\%$ vs TG IR-2hrs $57.3 \pm 3.5\%$; $p = \text{NS}$). (Figure 2 panel B)

Table 1 shows baseline HR, LVSP, LVEDP, $+dP/dt_{max}$ and $-dP/dt_{max}$ of the four experimental groups with 2-hour reperfusion following 30 minute-ischemia. Only HR evidenced a significant difference in the TG groups compared with WT mice. This positive chronotropic effect that was maintained throughout all the experiment is expected in transgenic mice.

During ischemia, LVEDP was significantly increased both in WT IR-2hrs (6.9 ± 1 mmHg) as in TG IR-2hrs (7.66 ± 0.7 mmHg) groups compared with their respective Sham groups (WT Sham-2hrs: 2.74 ± 0.1 mmHg and TG Sham-2hrs: 3.62 ± 0.2 mmHg) ($p < 0.05$). In turn, both groups with ischemia presented significantly elevated LVEDP with respect to their baseline values (WT IR-2hrs: Baseline 3.03 ± 0.2 mmHg vs. 30-min Ischemia 6.9 ± 1 mmHg; $p < 0.05$); (TG IR-2hrs: Baseline 2.64 ± 0.2 mmHg vs. 30-min Ischemia 7.66 ± 0.7 ; $p < 0.05$). No significant differences were encountered during ischemia or reperfusion in LVEDP and dP/dt_{max} between WT and TG mice.

Effects of $G_s\text{-}\alpha$ overexpression on ventricular function in chronic experiments

Figure 3 shows ejection fraction (EF) (panel A), SF (panel B) and FAC (panel C) values. Compared with the Sham group, I-28d groups showed a significant decrease of LVEF (WT: Sham-28d $82 \pm 2.4\%$ vs. I-28d $44 \pm 4\%$ and TG: Sham-28d $89 \pm 2\%$ vs. I-28d $42 \pm 3\%$), SF (WT: Sham-28d $44 \pm 2.6\%$ vs. I-28d $17 \pm 2\%$ and TG: Sham-28d $52 \pm 2.7\%$ vs. I-28d $16.7 \pm 1.2\%$) and FAC (WT: Sham-28d $50 \pm 2.6\%$ vs. I-28d $28.6 \pm 2.6\%$ and TG: Sham-28d $61.8 \pm 5\%$ vs. I-28d $26.5 \pm 2.5\%$) ($p < 0.05$). No significant differences were observed between WT and TG groups after 28 days of evolution.

Table 2 shows hemodynamic parameters obtained from left heart catheterization 28 after infarction. Heart rate was higher in TG compared with WT mice. A drop in $+dP/dt_{max}$ and $-dP/dt_{max}$ was observed in the ischemic groups. No significant differences were found in LVSP between groups. Conversely, LVEDP increased in the groups with ischemia with respect to their matching controls (WT: Sham-28d 3 ± 0.2 mmHg vs. I-28d 12 ± 2 mmHg and TG: Sham-28d 3.1 ± 0.3 mmHg vs. I-28d 9 ± 0.9 mmHg) ($p < 0.05$).

DISCUSSION

This study demonstrated that baseline sympathetic hyperactivity due to $G_s\text{-}\alpha$ protein overexpression coupled to the β -adrenergic receptor does not modify infarct size or left ventricular function after 30-minute ischemia and 2-hour reperfusion. It was also observed that protein $G_s\text{-}\alpha$ overexpression does not modify ventricular function in a model of chronic infarction with-

Fig. 2. Area at risk (Panel A) and infarct size (Panel B) of acute wild type (WT) and transgenic (TG) groups with 2-hour reperfusion after 30-minute ischemia. RA: Risk area. IA: Infarct area. IR: Ischemia/reperfusion. LV: Left ventricular. The parameters studied did not show significant differences.

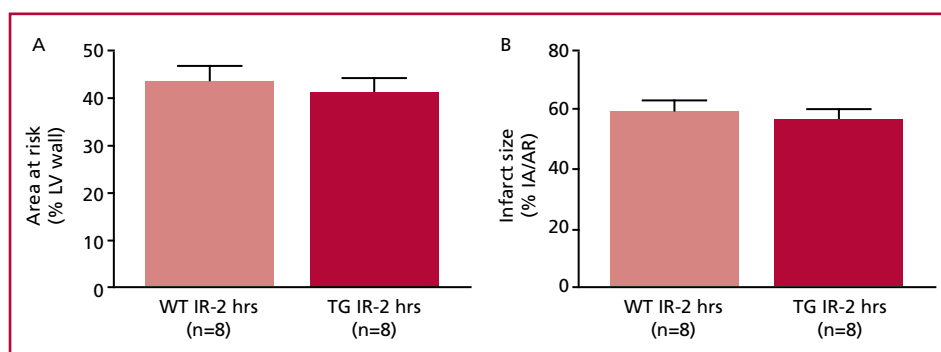


Table 1. Ventricular function and heart rate of wild-type (WT) and transgenic (TG) mice with 2-hour follow-up after reperfusion

		Groups	Baseline	30-min Isch.	5-min Rep	60-min Rep	120-min Rep
HR							
(bpm)	WT	Sham-2hrs	488±26	490±29	489±29	498±20	490±22
		IR-2hrs	459±22	461±14	481±11	487±15	489±15
	TG	Sham-2hrs	653±32*‡	649±32*‡	653±30*‡	652±31*‡	664±31*‡
		IR-2hrs	654±15*‡	666±15*‡	672±18*‡	645±17*‡	621±26*‡
LVSP							
(mmHg)	WT	Sham-2hrs	100±7	98±2	97±5	99±2	96±2
		IR-2hrs	90±3	70±5*	72±5*	80±4*	79±5
	TG	Sham-2hrs	88±4	89±4	89±3	87±4	89±5
		IR-2hrs	86±3	76±2	77±3	77±3	77±3
LVEDP							
(mmHg)	WT	Sham-2hrs	2.74±0.1	3.25±0.2	3.42±0.3	3.12±0.3	3±0.4
		IR-2hrs	3.03±0.2	6.9±1*†	5.68±2	4.14±1	4.21±1
	TG	Sham-2hrs	3.62±0.2	3.25±0.2	2.78±0.2	3.09±0.3	3.34±0.4
		IR-2hrs	2.64±0.2	7.66±0.7§¶	7.2±1.3	6.06±0.9	6.04±0.9
+dP/dtmax							
(mmHg/s)	WT	Sham-2hrs	8843±952	8330±238	8243±557	8566±416	7642±425
		IR-2hrs	6231±398	4561±471*	4619±503*	5338±423*	5139±582
	TG	Sham-2hrs	8258±713	8365±694	8237±807	8337±751	8284±732
		IR-2hrs	7889±544	7006±472	6386±614	6454±385	6423±508
-dP/dtmax							
(mmHg/s)	WT	Sham-2hrs	-6563±511	-6986±95	-6917±301	-7337±203	-6892±188
		IR-2hrs	-6039±350	-4190±456*	-4382±516*	-5237±464*	-5059±678
	TG	Sham-2hrs	-6746±220	-6600±419	-6882±233	-6712±247	-6775±408
		IR-2hrs	-6607±262	-5760±471	-5963±494	-6124±446	-6031±562

IR: Ischemia-reperfusion; Isch: Ischemia; Rep: Reperfusion; min: minutes; hrs: hours; HR: Heart rate; LVSP: Left ventricular systolic pressure; LVEDP: Left ventricular end-diastolic pressure; dP/dt: time derivative of the left ventricular pressure curve; bpm: beats per minute. Values are expressed as mean ± standard error. Statistical analysis: ANOVA for repeated measure followed by the Bonferroni test.

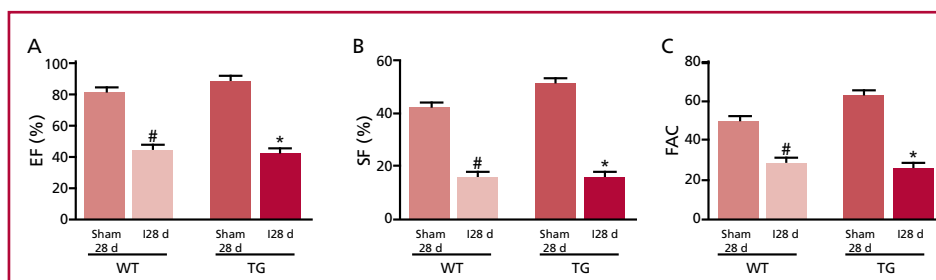


Fig. 3. Ejection fraction (%EF) (panel A), shortening fraction (%SF) (panel B) and fractional area changes (%FAC) (panel C) in WT and TG animals in Sham-28 day and I-28d. Statistical analysis: ANOVA for repeated measure followed by the Bonferroni's test.

WT: Wild-type mice; TG: Transgenic mice; I: Ischemia; d: Days. (#p<0,05 vs Sham-28d WT; *p<0,05 vs Sham-28d TG)

out reperfusion, assessed by echocardiography and cardiac catheterization. As shown in previous works, these transgenic mice have higher baseline cardiac function than non-transgenic animals, evidenced by increased heart rate and greater inotropic reserve (12, 15, 16) The increase in cardiac output implies greater myocardial oxygen consumption, that in the context of myocardial ischemia/reperfusion would result in greater injury, which is not reflected in the infarct size assessed in our experimental model. This suggests

that there are compensating mechanisms which avoid greater myocardial damage by β -adrenergic hyperactivity, at least in young animals and during an evolution period of one month. It would be interesting to evaluate these experiments with longer follow-up in which hearts have a higher degree of decompensation.

Contrary to the observations in this experimental project with young animals, G_s - α protein overexpression leads to progressive cardiomyopathy and decompensation in the hearts of older animals. (16) Pres-

Table 2. Left ventricular function and heart rate in WT and TG mice with 20-day follow-up after permanent regional ischemia

Groups		HR (bpm)	LVSP (mmHg)	LVEDP (mmHg)	+dP/dt _{max} (mmHg/s)	-dP/dt _{max} (mmHg/s)
WT	Sham-28d	462±6	100±3	3±0.2	8561±674	-7619±287
	I-28d	432±18*	74±4	12±2*	4269±285*	-4174±237*
TG	Sham-28d	485±17	94±4	3.1±0.3	8281±1212	-7551±260
	I-28d	540±7*†	84±5	9±0.9	6442±710	-5645±389

WT: Wild-type mice; TG: Transgenic mice; I: Ischemia-reperfusion; d: days; HR: Heart rate; bpm: beats per minute; LVSP: Left ventricular systolic pressure; LVEDP: Left ventricular end-diastolic pressure; dP/dt: Time derivative of the left ventricular pressure curve

ence of fibrosis, myocyte hypertrophy and abnormal ventricular function has been demonstrated as a consequence of chronic sympathetic stimulation. (16) For this reason, the animals in this study were used before the emergence of the baseline heart disease they would later develop.

Although TG mice had higher baseline heart rate and greater inotropic response, systolic ventricular pressure values were similar to those of WT animals. In this sense, it was previously shown these transgenic mice have slightly higher mean arterial pressure, suggesting that peripheral vascular resistance could be greater. (15) Although it was not our objective to comparatively study cardiac local or systemic sympathetic activation, it is possible that selective β_1 -adrenergic pathway activation is not the main cause of injury in ischemic cardiomyopathy, as would be the case of systemic neurohumoral activation. Further studies could provide new insight on the role of the sympathetic nervous system in myocardial damage and protection.

CONCLUSIONS

G_s - α protein overexpression does not increase infarct size nor modify left ventricular function in acute ischemia-reperfusion and in chronic infarction in a transgenic young mice model.

Conflicts of interest

None declared.

(See authors' conflict of interests forms on the web/Additional material.)

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