ORIGINAL ARTICLE

Left Ventricular Hypertrophy Inhibition, Normalization of the Contractile Response and Oxidative Stress in Experimental Hypertension

Inhibición de la hipertrofia ventricular izquierda, normalización de la respuesta contráctil cardíaca y estrés oxidativo en hipertensión experimental

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ABSTRACT

Introduction and objectives: Left ventricular hypertrophy secondary to hypertension has been perceived as a protective mechanism to reduce wall stress and prevent heart failure. However, its presence is paradoxically associated with increased cardiovascular morbidity and mortality. The aim of this study was to evaluate whether chronic antihypertensive treatment inhibits the development of left ventricular hypertrophy and normalize the impaired cardiac beta-adrenergic response, and its possible association with changes in myocardial oxidative metabolism.

Methods: Spontaneously hypertensive male rats (SHR, 2 months old) were divided into groups (ngroup = 18) according to (mg/kg, p.o): losartan 30 (L), hydralazine-11 (H), rosuvastatin 10 (R), carvedilol 20 (C), and water (control treatment). The control hypertension group consisted of 18 normotensive rats (Wistar-Kyoto, WKY). Systolic blood pressure (SBP) (plethysmography in awake animals) and body weight (BW) were measured periodically. The animals were sacrificed at 16 months and 50% of the hearts were mounted in a Langendorff system to measure contractility before and after beta-adrenergic stimulation [isoproterenol (Iso): 10-9 M, 10-7 M, and 10-5 M]. In the remaining hearts left ventricular weight (LVW) was measured and normalized by BW. Immunohistochemical expression of thioredoxin 1 (Trx-1), peroxyredoxin 2 (Prx-2) and glutaredoxin 3 (Grx-3) (antioxidant indicators) was quantified.

Results: Body weight was similar in all groups. Systolic blood pressure (mm Hg) was 154 ± 3 (L), 137 ± 1 (H), 190 ± 3 (R)**, 206 ± 3 (SHR)*, 183 ± 1 (C)**, and 141 ± 1 (WKY) (* $p < 0.05$ vs. L, H, WKY, ** $p < 0.05$ vs. L, H, WKY, SHR). LVW/BW was higher in SHR and R ($p < 0.05$) compared with L, H, C and WKY. In C, there was no correlation between hypertension and left ventricular hypertrophy. SHR, R and C evidenced baseline contractile depression vs. L, H and WKY. The response to 10-5 M Iso was similar in WKY and L, and reduced in C, H, R and SHR. The expression of Trx-1, Prx-2 and Grx-3 increased in C, H, R and L (average increase: 1.5-2 times; $p < 0.01$ vs. SHR and WKY).

Conclusions: Treatment with losartan, hydralazine, and carvedilol prevented the development of left ventricular hypertrophy. Losartan normalized the response to isoproterenol in SHR. Additional factors might participate in the development of left ventricular hypertrophy with impaired inotropic response to beta-adrenergic stimulation in hypertension. The increased expression of thioredoxins as a result of antihypertensive treatment suggests an additional benefit, increasing the antioxidant response against oxidative stress in hypertension.

Key words: Losartan - Rosuvastatin - Carvedilol - Hydralazine - Left Ventricular Hypertrophy - Oxidative Stress – Trx-1 – Grx-3 – Prx-2

RESUMEN

Introducción y objetivos: La hipertrofia ventricular izquierda secundaria a hipertensión arterial se ha interpretado como un mecanismo de protección para reducir el estrés parietal y prevenir la insuficiencia cardíaca. Sin embargo, paradójicamente, su presencia se acompaña de un incremento de la morbimortalidad cardiovascular. El presente estudio se llevó a cabo con el propósito de evaluar si el tratamiento antihipertensivo crónico inhibe el desarrollo de hipertrofia ventricular izquierda y revierte el deterioro de la respuesta betaadrenérgica cardíaca y su posible relación con cambios en el metabolismo oxidativo del miocardio.

Material y métodos: Ratas macho espontáneamente hipertensas (REH, 2 meses de edad) se distribuyeron en grupos (ngrupo = 18) según (mg/kg, v.o.): losartán 30 (L), hidralazina 11 (H), rosuvastatina 10 (R), carvedilol 20 (C), agua (control tratamiento). Control hipertensión: 18 ratas normotensas (Wistar-Kyoto, WKY). Periódicamente se registraron la presión arterial sistólica (PAS) (pletismografía, en animales despiertos) y el peso corporal (PC). Luego de 16 meses se practicó eutanasia. El 50% de los corazones se montaron en preparación de

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Langendorff para medir contractilidad preestímulo y posestímulo betaadrenérgico [isoproterenol (Iso): 10-9M, 10-7M, 10-5M]. En los corazones restantes se registró el peso del ventrículo izquierdo (PVI), que se normalizó por el PC. Se cuantificó la expresión inmunohistoquímica de tiorredoxina 1(Trx-1), peroxirredoxina 2 (Prx-2) y glutarredoxina 3 (Grx-3) (indicadores antioxidantes).

Resultados: Peso corporal: similar en todos los grupos. PAS (mm Hg): 154 ± 3 (L), 137 ± 1 (H), 190 ± 3 (R)**, 206 ± 3 (REH)*, 183 ± 1 (C)**, 141 ± 1 (WKY) (*p < 0,05 vs. L, H, WKY; **p < 0,05 vs. L, H, WKY, REH). El PVI/PC de REH y R fue mayor (p < 0,05) respecto de L, H, C y WKY. En C no se observó correlación entre hipertensión e hipertrofia ventricular izquierda. Grupos REH, R y C: mostraron depresión de contractilidad basal vs. L, H y WKY. Respuesta a Iso 10-5 M: similar en WKY y L; disminuida en C, H, R y REH. Expresión de Trx-1, Prx-2 y Grx-3: aumentó en C, H, R y L (1,5-2 veces promedio; p < 0,01 vs. REH y WKY).

Conclusiones: El tratamiento con losartán, hidralazina y carvedilol previno el desarrollo de hipertrofia ventricular izquierda. El losartán normalizó la respuesta al isoproterenol en REH. Factores adicionales participarían en el desarrollo de hipertrofia ventricular izquierda con deterioro de la respuesta inotrópica a la estimulación betaadrenérgica en hipertensión. El aumento en la expresión de tiorredoxinas por tratamientos antihipertensivos sugiere un beneficio asociado, aumentando la respuesta antioxidante frente al estrés oxidativo en hipertensión.

Palabras claves: Losartán - Rosuvastatina - Carvedilol - Hidralazina - Hipertrofia ventricular izquierda - Estrés oxidativo - Tiorredoxina 1 - Glutarredoxina 3 - Peroxirredoxina 2.

Abbreviations

BW	Body weight	**LVW**	Left ventricular weight
C	Carvedilol	**Prx-2**	Peroxyredoxin 2
Grx-3	Glutaredoxin 3	**PW**	Pulmonary weight
H	Hydralazine	**R**	Rosuvastatin
HMG-CoA	Hydroxymethylglutaryl-coenzyme A	**ROS**	Reactive oxygen species
HF	Heart failure	**SHR**	Spontaneously hypertensive rats
L	Losartan	**Trx-1**	Thioredoxin 1
LVH	Left ventricular hypertrophy	**WKY**	Wistar-Kyoto

INTRODUCTION

Extended lifespan, as a result of pharmacological and technological development has favored the increase of diseases such as heart failure (HF).

Left ventricular hypertrophy (LVH) secondary to hypertension has been interpreted as a protective mechanism to reduce wall stress and prevent HF. However, the presence of LVH is paradoxically accompanied by an increase in cardiovascular morbidity and mortality.

The aim of this study was to evaluate in spontaneously hypertensive rats (SHR, one of the inbred strains most widely used as animal model to study human essential hypertension) whether chronic treatment with drugs commonly used to treat hypertension (either hypotensive or not agents), and which act on different physiological mechanisms, is capable of inhibiting the development of LVH reverting impaired cardiac beta-adrenergic response independently of blood pressure normalization, and whether the possible effects correlate with changes in myocardial oxidative metabolism.

Heart failure is a complex clinical syndrome as a result of functional and/or structural alterations, whereby the heart is unable to maintain an adequate cardiac output in the presence of different tissue oxygen requirements.

Hypertension (HT) is one of the most important risk factors for the development of HF and LVH, and the presence of the latter increases up to 6 times the chance of developing HF. (1, 2)

It has been postulated that LVH has a protective role in the preservation of contractile function and prevention of HF. The Framingham study showed that LVH, according to the anatomical pattern developed, is an independent risk factor for general and cardiovascular morbidity and mortality including adverse cardiovascular events, such as HF, atherosclerosis, stroke and/or death. (3, 4) Epidemiological findings raise the need to resolve the issues regarding the role of LVH in the development and progression of HF.

It has been suggested that similar to the important role of reactive oxygen species (ROS) in remodeling and LVH, (5) antioxidants such as glutathione, antioxidant enzymes of the thioredoxin family and others such as superoxide dismutase and catalase, may have a protective effect reducing or counterbalancing the deleterious action of ROS. (6)

Of note, low concentrations of ROS induce Trx-1 expression but at high concentrations inactivate Trx-1 through decoupling secondary to nitric oxide (NO) and ROS imbalance. Thus, Trx-1 inactivation with the ensuing increase in ROS levels may participate in diverse responses such as hypertrophy or apoptosis. (7, 8) It has also been reported that Trx-1 reduces cardiac angiotensin II- induced ventricular hypertrophy. (9)

This study evaluates whether chronic antihypertensive treatment inhibits the development of LVH with recovery of impaired cardiac beta-adrenergic response, and its possible association with changes in myocardial oxidative metabolism. The effect of different drugs used as antihypertensive agents was evaluated: losartan (indirect hypotensive agent blocking angiotensin II receptor, type 1), hydralazine (direct hypotensive agent due to its vasodilating effect), ro-

suvastatin [lipid-lowering statin that blocks the conversion of hydroxymethylglutaryl-coenzyme A (HMH-CoA) to mevalonate] (10, 11) and carvedilol (beta blocker with antioxidant, hypotensive and antihypertrophic activity) (12, 13)

METHODS

Male spontaneously hypertensive rats (SHR, 2-month old) were distributed in groups (group n = 18) according to (mg/kg p.o.) of: losartan 30 (L), hydralazine 11 (H), rosuvastatin 10 (R), carvedilol 20 (C), and water (control treatment). Normotensive (Wistar-Kyoto, WKY) rats (n = 18) were used as control hypertension. All animals were housed at the ININCA animal house , with 12-12 hour light–darkness cycles, at a temperature of 21± 2 °C and fed a balanced extruded chow containing normal sodium and 16-18% proteins (Cooperación-Argentina) according to the Canadian Council on Animal Care (1980-1984) (Guide to the care and use of experimental animals, 2 vol., Ottawa, Ont: CCAC) recommendations.

Body weight was measured on a weekly basis. Blood pressure was recorded every two weeks by tail pletismography using a NIBP controller module (ADInstruments) in conjunction with a PowerLab system (ADInstruments). Recordings were stored and analyzed using LabChart software (ADInstruments).

Euthanasia

At the end of treatment (16 months) all study animals were sacrificed with an overdose of pentobarbital (40 mg/kg i.p).

Contractile function response to beta-adrenergic stimulation

In each group, 50% of the hearts were mounted in a modified Langendorff perfusion system. The complete procedure lasted less than 1 minute. The heart was perfused with Krebs-Henseleit buffer (mM: NaCl 118.5; KCl 4.7. NaHCO3 24.8; KH2PO 1.2; MgSO4 1.2; CaCl2 2.5; glucose 10) at pH =7.43-7.47, bubbled with 95% O2 at 37°C. Two electrodes were placed on the heart and connected to a pacemaker providing a stimulus of 175 beats / min.

A latex balloon was inserted into the LV and connected through a polyethylene tube to a Deltram II pressure transducer (Utah Medical System) and this to a computer via an analogue-digital converter. This equipment was used for real-time acquisition and storage of left ventricular pressure and its derivative (dP/dt+).

Systolic ventricular pressure and maximum dP/dt+ (dP/dt +) (indicating maximum velocity of contractile force development) in response to isoproterenol 10-9, 10-7 and 10-5 M were determined.

Thioredoxin immunohistochemical expression

The remaining hearts were dissected and the left ventricles were weighed (LVW) and conventionally processed for immunohistochemical assessment. Specific antiredoxin antibodies were used: anti-thioredoxin-1, anti-glutaredoxin-3 and anti-peroxiredoxin-2 (anti-Trx-1, anti-Grx-3, anti-Prx-2, AbcamInc, Cambridge, Ma, USA). Left ventricular weight was normalized by body weight (BW).

Nuclear Trx-1 and Grx-3 expression and interstitial Prx-2 expression were quantified as indicators of antioxidant response.

Both lungs were weighed (PW) and the values were normalized by BW (PW/BW)

Statistical analysis

ANOVA followed by the Bonferroni test was used to assess differences between factors of interest using SPSS 14.0 statistical software package. Statistical difference was considered for $p < 0.05$.

Ethical considerations

The protocol was performed according to the recommendations of the Canadian Council on Animal Care (1980-1984) (Guide to the care and use of experimental animals. 2 vols. Ottawa, Ont.: CCAC).

RESULTS

Blood pressure

In the SHR group, BP progressively increased since study onset (2 month-old rats) and reached maximum values after 4 months. The groups treated with losartan or hydralazine did not evidence this increase and their BP values were similar to those observed in the WKY group (Figure 1).

Conversely, rosuvastatin did not modify BP, whereas, treatment with carvedilol had a modest hypotensive effect throughout the study period. In the WKY group, BP increased slightly with age.

Body weight

Body weight was similar in all the groups at onset and throughout the whole study. A gradual and parallel increase was seen in all the groups during the period of life analyzed (Figure 2).

Left ventricular weight-body weight ratio

The SHR and R groups showed increased LVW-BW ratio (LVW/BW) compared with the other groups (Figure 3). Also PW normalized by BW (PW/BW) increased in SHR and R groups ($p < 0.05$) compared with the other groups (L 3.21±0.12, H 3.63±0.1, R 5.8±0.43, SHR 5.64±0.31, WKY 3.6±0.31, and C 3.91±0.27).

Contractile response to beta-adrenergic stimulation with isoproterenol

SHR, R and C groups evidenced lower baseline contractility compared to the WKY group.

The WKY and L groups had a similar response to increasing doses of isoproterenol stimulation. Conversely, SHR, R and C groups decreased the response to isoproterenol stimulation (Figure 4).

Thioredoxin immunohistochemical expression

Treatment with H, L, C and R resulted in increased specific marker for Trx-1 (Figure 5, left panel and upper right panel) and for Grx-3 (Figure 5, left panel and lower right panel) (nuclear) compared to that observed in non-treated SHR and WKY animals. In turn, Trx-1 expression was lower in the SHR group than in the WKY group (Figure 5 left panel and upper right panel).

Prx-2 expression (interstitial) showed a pattern similar to that observed for Trx-3 (Figure 5, left panel

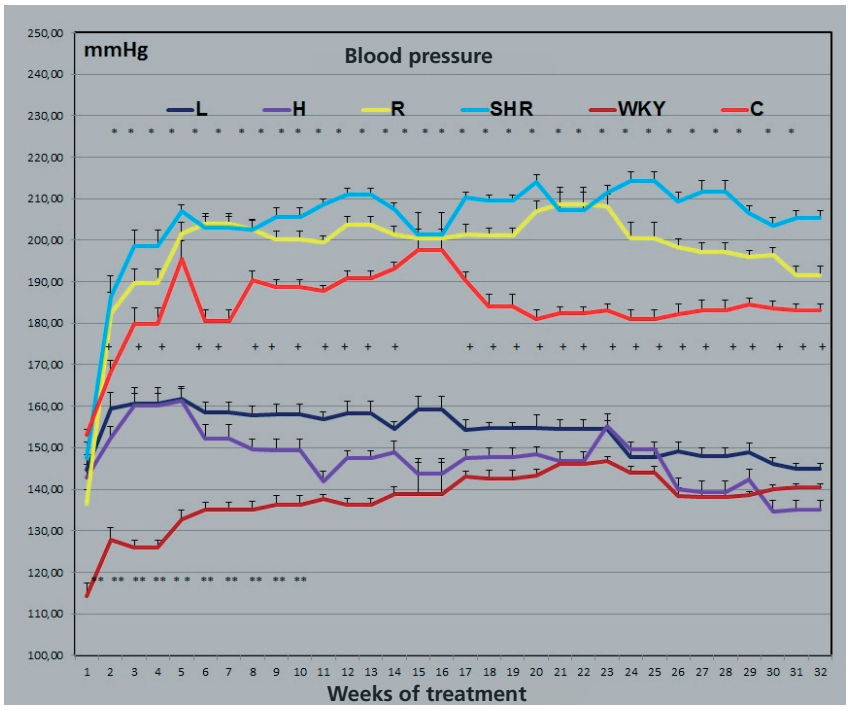

Fig. 1. Blood pressure as a function of study time. L: Losartan, H: Hydralazine, R: Rosuvastatin, C: Carvedilol, SHR: Water (control treatment). WKY: Normotensive Wistar-Kyoto rats.
* P < 0.05 vs. WKY, L, H; + P < 0.05 vs. SHR, L, H, WKY; ** P < 0.05 vs. L, H, R, SHR, C.

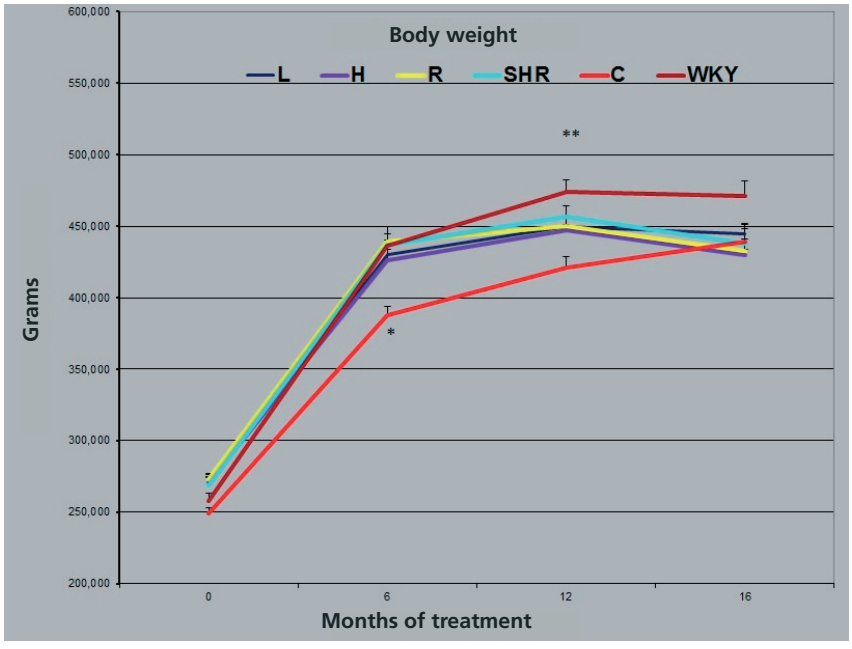

Fig. 2. Body weight as a function of study time. L: Losartan, H: Hydralazine, R: Rosuvastatin, C: Carvedilol, SHR: Water (control treatment). WKY: Normotensive Wistar-Kyoto rats. * P < 0.05 vs. R; ** P < 0.05 vs. C.

and middle right panel).

DISCUSSION

Absence of the hypotensive effect of rosuvastatin is not surprising since it is a lipid-lowering drug whose pharmacological action consists in blocking HMG-CoA conversion into mevalonate. It is thought that the BP increase observed in the WKY group as a function of study time would be associated with the natural aging process of the animal.

Left ventricular adaptation to high BP during long periods, without weight increase as observed in the carvedilol group, with depressed ventricular function both at baseline and in response to beta-adrenergic

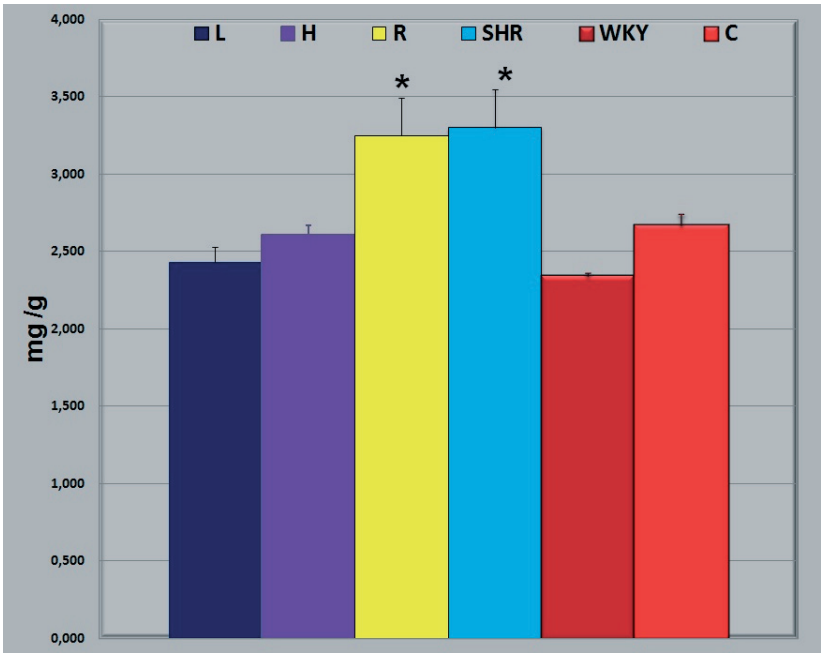

Fig. 3. Left ventricular weight relative to body weight. L: Losartan, H: Hydralazine, R: Rosuvastatin, C: Carvedilol, SHR: Water (control treatment). WKY: Normotensive Wistar-Kyoto rats. * p < 0.05 vs. L, H, WKY and C.

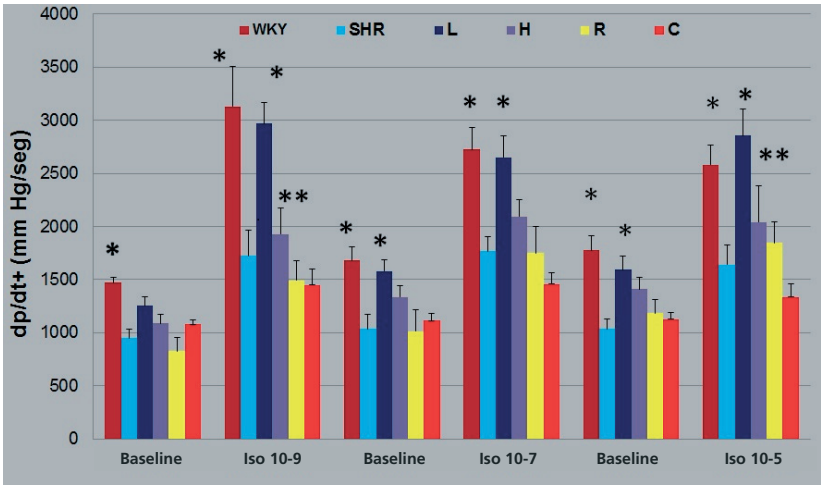

Fig. 4. Myocardial contractile function in response to beta-adrenergic stimulation with isoproterenol (Iso). L: Losartan, H: Hydralazine, R: Rosuvastatin, C: Carvedilol, SHR: Water (control treatment). WKY: Normotensive Wistar-Kyoto rats. * P < 0.05 vs. SHR, R, C; ** p < 0.05 vs. C; * p < 0.05 vs. SHR, H, R, C

stimulation, and without HF, confirms the role played by neuroendocrine activation in the development of HF. It also challenges the traditional concept of the indispensable role of cardiac hypertrophy as a protective mechanism, opening the debate about the involvement of increased wall stress as a necessary factor in the development of ventricular hypertrophy.

These results suggest that LVH, expressed as relative weight increase (LVW/BW) in response to sustained increase in afterload, would not be a necessary adaptive phenomenon to prevent HF and that the increase of ventricular weight would be a determining factor in HF development as observed in SHR and R groups.

Decreased antioxidant activity (evidenced by lower nuclear expression of Trx-1) in the SHR group, would allow assuming that the decreased antioxidant activity participates in LVH and HF.

Thioredoxin 1 participation in ventricular remodeling

It has been claimed that just as the generation of reactive oxygen species (ROS) plays an important role in remodeling and LVH, antioxidants such as glutathione and antioxidant enzymes as thioredoxins (Trx-1, Grx-3, Prx-2) may have a protective effect and attenuate or counterbalance the deleterious action of ROS. (5, 6)

It is remarkable that at low concentrations ROS induce Trx-1 expression, but at high concentrations inactivate Trx-1 through decoupling secondary to an imbalance between nitric oxide (NO) and ROS. Thus,

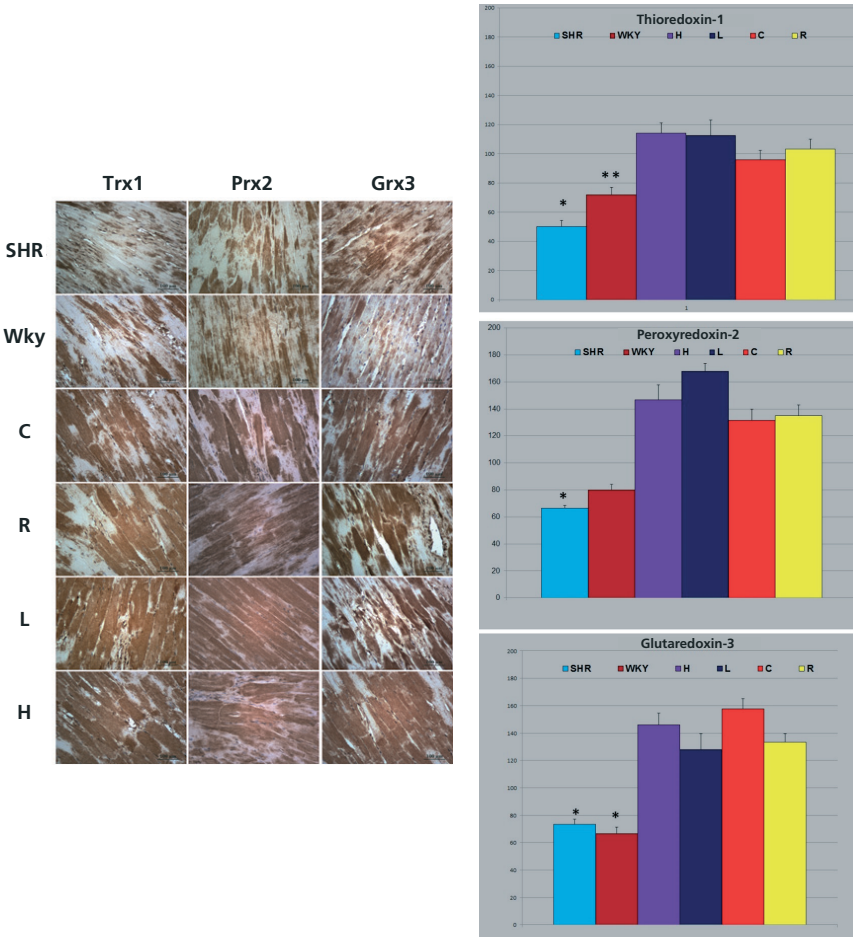

Fig. 5. Effect of study drugs on the myocardial immunohistochemical expression of thioredoxins Trx-1, Prx-2 and Grx-3. Left panel: Micrograph of histological sections showing the immunohistochemical expression of thioredoxin 1 (Trx-1), peroxyredoxin 2 (Prx-2) and glutaredoxin 3 (Grx-3). There is a clear increase in positive areas for Trx-1, Prx-2 and Grx-3. Right panel: Results quantified for each thioredoxin according to the experimental group. For Trx-1: * $p < 0.05$ vs. WKY, L, H, R, C; ** $P < 0.05$ vs. L, H, C, R. For Prx-2 and Grx-3: * $p < 0.05$ vs. L, H, R, C. L: Losartan, H: Hydralazine, R: Rosuvastatin, C: Carvedilol, SHR: Water (control treatment). WKY: Normotensive Wistar-Kyoto rats. Trx-1: Thioredoxin 1; Prx-2: Peroxyredoxin 2; Grx-3: Glutaredoxin 3.

ROS-mediated Trx-1 activity may participate in diverse responses such as hypertrophy or apoptosis. (14) Increased Trx-1 activity in all treated groups would not be related to hypertrophy, since it was observed independently of hypertrophy and/or HF.

Thioredoxin 1 and cardiac hypertrophy

It is known that low concentrations of ROS promote cell development, but also that the antioxidant action of Trx-1 can attenuate or modify it (15, 16).

Different tests with transgenic mice overexpressing Trx-2 or genetically deficient in Trx-1 confirm the involvement of this enzyme as a modulator of myocardial hypertrophy. (17) The probable mechanism by which Trx-1 inhibits LVH could be by blocking the Ras-MAPK pathway (18), although other mechanisms and signaling pathways regulated by Trx-1 might be involved.

Heart failure is a complex neurohumoral and inflammatory syndrome (19) where ROS production is increased and antioxidant activity decreased (20-22).

There are various mechanisms participating in contractile depression associated with HF. (23, 24) In LVH and HF a vicious circuit has been shown, whereby ROS formation increases as a result of mitochondrial dysfunction initially induced by ROS. (25, 26)

CONCLUSIONS

These experimental results show dissociation between LVH expressed by increased ventricular weight, hypertension and contractile response to beta-adrenergic stimulation, and HF.

The increase in antioxidant response induced by all the drugs used in the study (including the rosuvastatin group) independently of LVH and HF indicates that the increased antioxidant defense would be insufficient to prevent HF, conferring it a secondary role in the development of LVH and HF.

Conflicts of interest

None declared.
(See author´s conflicts of interest forms in the web / Supplementary Material)

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