ORIGINAL ARTICLE

Thioredoxin-1 Reduces Infarct Size But Does Not Improve Postischemic Ventricular Dysfunction

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ABSTRACT

Background
Thioredoxin-1 is a potent endogenous antioxidant involved in myocardial protection from ischemic/reperfusion injury. However, it is unknown whether this protection is preserved in middle age or whether exist a dissociation between the effect on ventricular function and infarct size.

Objective
The purpose of this study was to compare infarct size and ventricular function in young and middle-aged transgenic mice overexpressing thioredoxin-1 with their corresponding wild-type controls.

Methods
Isolated hearts of 3-month (young) and 12-month (middle-age) FVB male mice were submitted to 30 minutes of global ischemia and 120 minutes of reperfusion using the Langendorff technique. Four experimental groups were considered: young wild-type, middle-aged wild type, young thioredoxin-1 and middle-aged thioredoxin-1. Left ventricular function was assessed and infarct size was measured with triphenyl tetrazolium.

Results
Ventricular function showed no significant differences between the studied groups. However, young thioredoxin-1 mice reduced infarct size (27.6%±3.5% vs. 42.9%±6.1% young wild-type mice); conversely, the middle-aged thioredoxin-1 group was not significantly different from its wild-type control (49.1%±6.4% vs. 52.6%±5.2%).

Conclusions
Results suggest that young mice overexpressing thioredoxin-1 reduce infarct size, but without changes in ventricular function. Moreover, the protective antioxidant effect is abolished in middle-aged transgenic mice.

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Key words > Myocardial Ischemia - Reperfusion - Myocardial infarction - Ventricular function - Antioxidants - Thioredoxin

Abbreviations >

+dP/dt	Positive first derivative of left ventricular pressure	**LVEDP**	Left ventricular end-diastolic pressure
CPP	Coronary perfusion pressure	**NTG**	Non-transgenic
I/R	Ischemia/reperfusion	**ROS**	Reactive oxygen species
LVDP	Left ventricular developed pressure	**TRX**	Thioredoxin

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INTRODUCTION

Thioredoxin is a powerful antioxidant, and TRX-1 (12 kDa) has specific antiapoptotic, (1) anti-inflammatory, (2) and protective effects against ischemia/reperfusion (I/R) injury. (3) Aota et al, (4) showed that in I/R injury, human recombinant TRX administration reduced the incidence of reperfusion arrhythmias. Nakamura et al, (5) found that TRX inactivity in patients submitted to cardiopulmonary bypass surgery had a detrimental effect on I/R injury. Similarly, Tao et al. (6) showed that in vivo TRX-1 administration evidenced significant cardioprotective effects, reducing apoptosis and infarct size. It is therefore clear that TRX-1 confers protection from I/R.

On the other hand, there is an important experimental support demonstrating that reactive oxygen (ROS) and nitrogen species increase significantly in aging animals, enhancing I/R injury. (7, 8) It has also been shown that infarct size and apoptosis increase in old animals as a consequence of physiologic TRX inactivity. (9, 10) Although oxidation processes start at birth, it is in middle age that they reach sufficiently high levels to trigger deleterious mechanisms on different cellular components, (11) allowing increased ROS to modify the expression and/or activity of different proteins. (12-14) However, it is unknown whether during middle age, TRX-1 has expression and/or activity alterations that might induce changes in infarct size as a result of I/R injury.

The purpose of this work was to assess infarct size and ventricular function behavior in young and middle-aged transgenic mice overexpressing TRX-1, comparing results with those corresponding to young and middle-aged non-transgenic (NTG) controls.

METHODS

Young (3-month old, n=6) and middle-age (12-month old, n=7) male FVB mice overexpressing TRX-1 protein and their corresponding NTG controls (n=7 and n=8, respectively) were used. Transgenic mice were generated using the myosin-α heavy chain promoter to achieve specific cardiac overexpression (courtesy of Prof. Junichi Sadoshima, Department of Cell Biology and Molecular Medicine, University of Medicine and Dentistry of New Jersey (UMDNJ)].

Ventricular function assessment

Hearts were perfused according to the modified Langendorff technique and were submitted to a protocol consisting of 30-minutes global ischemia followed by 120 minutes of reperfusion. After 3-or 12-month follow-up, mice were anesthetized with an intravenous combination of pentobarbital (150 mg/kg) and sodium heparin (500 IU/kg) (approved by the Institutional Animal Care and Use Committee (IACUC), Federal Administration's Unique Key Documentation (CUDAP), File No-UBA: 0037016/2010. Following anesthesia, the thorax was opened and the aorta was isolated and immediately cannulated with a 21 G cannula. The heart was then placed in a Langedorff system and perfused with Krebs-Henseleit solution (118.5 mM NaCl, 4.7 mM KCl, 24.8 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM MgSO4, 1,5 mM CaCl2 and 10 mM glucose), continuously bubbled with 95% O2 and 5% CO2 at pH 7.4 and 37 °C to keep the the solution oxigenated and at a physiological pH.

A latex balloon connected by a thin plastic catheter (P50) to a pressure transducer (Deltram II, Utah Medical System) was inserted into the left ventricle through the left atrium. The balloon was filled with aqueous solution to attain a diastolic pressure of approximately 10 mmHg.

Two electrodes were placed at the base of the atria and connected to a pacemaker to maintain constant heart rate throughout the experiment. Coronary perfusion pressure (CPP) was registered with a pressure transducer connected to the perfusion line. All hearts were perfused at constant flow. Real-time records of left ventricular pressure and CPP were acquired using a data acquisition program. Left ventricular developed pressure (LVDP) was calculated as the difference between left ventricular maximum systolic pressure and left ventricular end diastolic pressure (LVEDP). In addition, LVEDP and the maximum velocity of left ventricular pressure increase or positive first derivative of pressure with respect to time (+dP/dt) were measured.

Infarct size measurement

Hearts were frozen after ventricular function assessment and then cut into 1 mm thick sections which were incubated in 1% 2, 3, 5 triphenyl tetrazolium chloride (TTC) at pH 7.8 and at 37º C during 20 minutes. With this technique, viable tissue stains red, while the non-stained area corresponds to the infarct zone. Sections were scanned and the area corresponding to the ventricular wall and infarcted areas were measured using computerized planimetry (Image Pro Plus Image, version 4.5). Infarct size was expressed as percentage of left ventricular area.

Statistical analysis

The Kolmogorov-Smimov test was used to analyze normal data distribution. Data were expressed as mean ± standard error. Groups were compared using one-way ANOVA with Bonferroni's post-hoc test for multiple comparisons. A p value < 0.05 was considered as statistically significant.

RESULTS

Table 1 illustrates mean values and the corresponding dispersion of baseline hemodynamic variables, which are normal for the mouse species.

Figure 1 shows left ventricular function parameters. Panel A, representing left ventricular developed pressure (LVDP, mm Hg) behaviour, shows that baseline values were similar among the groups studied (young NTG: 74.2 ± 2.3 mm Hg, middle-aged NTG: 80.5 ± 10.5 mm Hg, young TRX-1: 87.7 ± 11.6 mm Hg and middle-aged TRX-1; 87.1 ± 7.8 mm Hg). At 30 minutes of reperfusion there was a decrease in the contractile state, evidenced by reduced LVDP compared to its pre-ischemic values, but with no significant differences among the four groups (young NTG: 17.9 ± 4.7 mm Hg, middle-aged NTG: 22.1 ± 7.8 mm Hg, young TRX-1: 30.6 ± 3.5 mm Hg and middle-aged TRX-1; 29.2 ± 7.7 mm Hg). Panel B shows maximum velocity of pressure increase (+dP/dt, mm Hg/s), which is another contractility index. Baseline values were similar among the four groups studied (young NTG: 2951 ± 206 mm Hg/s, middle-aged NTG: 2749 ± 717 mm Hg/s, young TRX-1: 2927 ± 701 mm Hg/s and middle-aged TRX-1; 3228 ± 439 mm Hg/s) and

during reperfusion there was a fall in the contractile state without significant differences among groups (young NTG: 803 ± 196 mm Hg/s, middle-aged NTG: 1285 ±357 mm Hg/s, young TRX-1: 1285 ± 428 mm Hg/s and middle-aged TRX-1; 1196 ± 339 mm Hg/s). Panel C represents left ventricular end-diastolic pressure (LVEDP) behavior. No significant differences among groups were observed in baseline conditions (young NTG: 7.8 ± 1.1 mm Hg, middle-aged NTG: 8.8 ± 2.8 mm Hg, young TRX-1: 7.1 ± 0.8 mm Hg and middle-aged TRX-1; 10.4 ± 1.1 mm Hg). As expected, at 30 minutes of reperfusion there was a marked increase of myocardial stiffness without significant differences among groups (young NTG: 18.5 ± 5.8 mm Hg, middle-aged NTG: 34.2 ± 8.5 mm Hg, young TRX-1: 18.4 ± 7.1 mm Hg and middle-aged TRX-1; 23.3 ± 6.6 mm Hg).

Figure 2 shows infarct size behavior. Infarct size produced by 30 minutes of ischemia followed 120 minutes of reperfusion was 42.9 ± 6.1% in young NTG animals. On the other hand, in middle-aged NTG mice there was a slight, though not significant trend towards increased injury (52.6 ± 5.2%). Hearts of young mice with TRX-1 overexpression had reduced infarct size compared to their NTG controls (27.6% ± 3.5%, p ≤ 0.05 vs. young NTG). However, this protection was not evident in middle-aged TRX-1 mice (49.1 ± 6.4%). Thus, overexpression of TRX-1 decreases infarct size in young mice, but does not produce changes in middle-aged animals.

Table 1. Mean baseline hemodynamic variables

	Hemodynamic variables		
	Coronary flow	Heart rate	Coronary perfusion pressure
Basal	4.0 ± 0.2 ml/min	472 ± 30.2 beats/min	73.1 ± 3.1 mm Hg

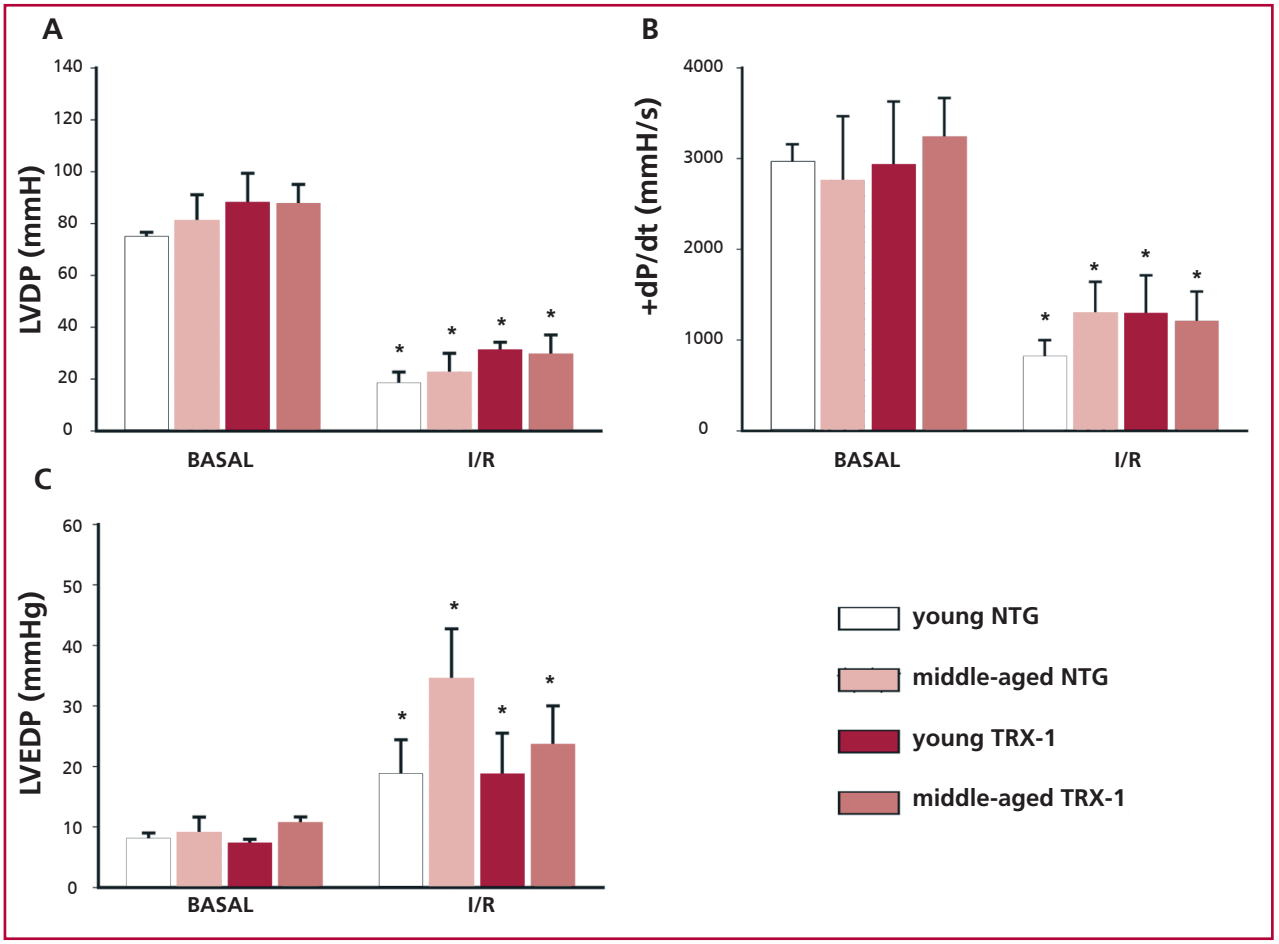

Fig. 1. Contractile (panels A and B) and myocardial stiffness (panel C) variables. Panel **A** shows basal left ventricular developed pressure (LVDP) and decreased postischemic contractility, with no significant differences among groups either in basal conditions or after ischemia/reperfusion. Panel **B** shows basal maximum velocity of pressure development (+dP/dt) which similarly to LVDP decreases after ischemia with no significant differences among groups either in basal conditions or after ischemia/reperfusion. Panel **C** depicts left ventricular end diastolic pressure (LVEDP) showing increased stiffness after ischemia and as with the systolic component, no significant difference among groups either in basal conditions or after ischemia/reperfusion. * p < 0,05 vs. basal value of each group. NTG: Non- transgenic. TRX-1: Thioredoxin-1. I/R: Ischemia/reperfusion.

DISCUSSION

This study provides experimental evidence that TRX-1 overexpression in young transgenic mice protects the myocardium against ischemia/reperfusion injury, since these animals showed reduced infarct size after 30 minutes of ischemia followed by a 120-minute reperfusion period. This protection, however, was abolished in middle-aged TRX-1 mice. Moreover, myocardial protection against infarct size was not accompanied by ventricular function recovery during reperfusion in none of the groups studied.

Tao et al., (15) in a mice model subjected to 30 minutes of ischemia and intraperitoneal human-recombinant TRX-1 administered at reperfusion, demonstrated that the TRX-1 protective effect on infarct size and apoptosis is produced by reducing oxidative/nitrative stress. On the other hand, Turoczi et al. (3) used for the first time a murine model of TRX-1 overexperssion to study I/R injury and found that in hearts submitted to a 30-minute ischemia and 120-minute reperfusion protocol, infarct size was significantly reduced in animals with TRX-1 overexpression. They also reported an improvement postischemic ventricular function in transgenic mice. In this point, their results differ from those of our study, where there was a decrease in infarct size, but without changes in left ventricular function. The divergent results could be attributed to the different animal models employed in these studies. In Turoczi et al study, (3) where an in vitro isolated working heart with antegrade perfusion was used, loading conditions were not kept constant and their changes could have impacted on ventricular function. Conversely, the retrograde Langendorff perfusion used in our study, where the isolated heart is not ejective but isovolumic, maintained constant loading conditions (preload and afterload) during the experimental protocol, thus allowing a more accurate assessment of contractility. In addition, in the isolated heart model presented here, heart rate, one of the major determinants of contractile state, was kept constant throughout the experiment. These differences in the experimental model could therefore account, at least partially, for differences in results.

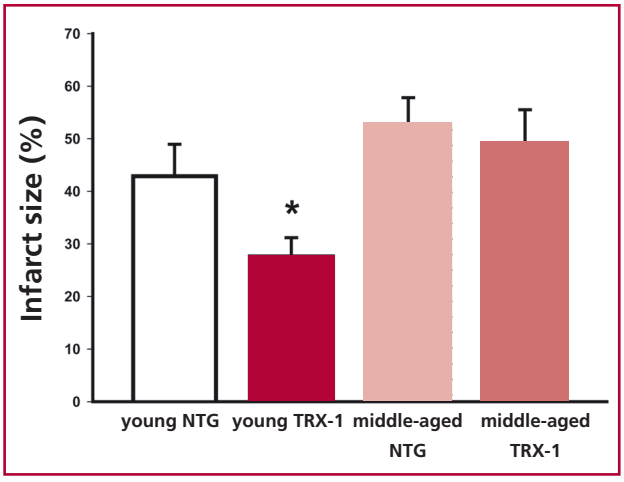

Fig. 2. Infarct size in young (young NTG and young TRX-1) and middle-aged (middle-aged NTG and middle-aged TRX-1) mice expressed as percentage of total left ventricular area. A significant reduction in infarct size is observed in young mice overexpressing thioredoxin-1; however, in middle-aged mice overexpressing the ontioxidant, infarct size is comparable to control. * $p < 0,05$ vs. young NTG. NTG: Non- transgenic. TRX-1: Thioredoxin-1.

Alternatively, lack of ventricular function protection could be explained by areas of myocardial stunning (reversible postischemic ventricular dysfunction in the absence of necrosis) in the border zone of the infarcted area, which is associated with the phenomenon of I/R injury. (16) In this sense, it is known that in the presence of infarction, there is a certain degree of postischemic dysfunction (stunned myocardium) in adjacent areas, which recovers after 72 hours of reperfusion. (17) Thus, acute changes in infarct size will not significantly influence ventricular function recovery, as can be seen in the model used in this study. This could probably be another reason why postischemic ventricular function improvement could not be obtained, since model limitations prevented experimental monitoring during the 48-72-hour period required to assess ventricular function recovery. (18-21) The controversy regarding dissociation in protection against infarct size and ventricular function could be due to different in vivo or in vitro experimental infarct models, with global or regional ischemia and with infarct assessment at different reperfusion times.

Consistent with our findings, Samuel et al., (22) using type-1 diabetic rats to assess recovery from myocardial infarction by intramyocardial TRX-1 administration, found significant improvement of echocardiographic functional parameters 4 weeks after infarction and treatment initiation. These authors clearly demonstrated the protective role of TRX-1, involving not a single mechanism but many intracellular pathways, and was involved in severalfunctions. It is noteworthy that they found improved ventricular function 4 weeks after the intervention, whereas reduced fibrosis and oxidative stress were already evident at 4 days.

It is therefore clear that increased TRX-1 in the organism, generated by exogenous administration or overexpression models of the protein, has an important role mediating cardioprotection in I/R injury. This leads, among other benefits, to significant infarct size reduction compared to their corresponding controls.

However, protection is abolished in middle-aged transgenic mice, which extrapolated to the clinical setting might explain contradictory results of antioxidant therapies in patients with ischemic cardiomyopathy. Consistent with this hypothesis, several studies have demonstrated that the levels of expression of some proteins are altered during aging (23, 24) as well as during stress conditions, such as in I/R injury. (25, 26) Moreover, these protein expression and functional

changes have been associated with increased ROS generation which becomes evident during aging, but is already present in middle age. (26)

Zhang et al. showed that TRX-1 activity decreases in 20-month old mice due to post-translational modifications such as nitration. The novelty of our finding is that at 12 months, when the detrimental effects of pro-oxidant mechanisms associated to aging are not fully established yet,, we found TRX-1 functional alterations evidenced by lack of protection against infarction. A possible explanation for these results is that the protein is nitrated as a consequence of increased age. However, further studies are needed to test this hypothesis.

CONCLUSIONS

The present results suggest that TRX-1 protects against I/R injury reducing infarct size in young mice overexpressing this protein compared with NTG mice. Conversely, in middle-aged mice with TRX-1 overexpression, protection is abolished. This finding could possibly explain failure of some antioxidant therapies to treat ischemic cardiomyopathy. Consequently, antioxidant action mechanisms should be further studied, especially since middle age.

RESUMEN

La tiorredoxina-1 no atenúa la disfunción ventricular posisquémica a pesar de disminuir el tamaño del infarto

Introducción

La tiorredoxina-1 es un potente antioxidante endógeno que participa en la protección miocárdica frente a la lesión por isquemia/reperfusión. Sin embargo, no se ha estudiado si esta protección se mantiene en la edad media de la vida y si se produce una disociación entre el efecto sobre la función ventricular y el infarto de miocardio.

Objetivo

Evaluar el tamaño del infarto y la función ventricular en ratones transgénicos jóvenes y de edad media que sobreexpresan tiorredoxina-1, comparándolos con sus respectivos controles no transgénicos.

Material y métodos

Se utilizaron corazones aislados de ratones FVB machos de 3 (jóvenes) y 12 (edad media) meses que fueron sometidos a una isquemia global de 30 minutos seguida por 120 minutos de reperfusión según la técnica de Langendorff. Se conformaron cuatro grupos experimentales: no transgénico jóvenes, no transgénico edad media, tiorredoxina-1 jóvenes y tiorredoxina-1 edad media. Se evaluó la función del ventrículo izquierdo y también se midió el tamaño del infarto (trifenil tetrazolio).

Resultados

La función ventricular no mostró cambios significativos entre los grupos estudiados. Sin embargo, se observó una disminución del tamaño del infarto en ratones tiorredoxina-1 jóvenes (27,6% ± 3,5% vs. 42,9% ± 6,1% en no transgénico jóvenes); en cambio, el grupo tiorredoxina-1 de edad media no presentó cambios frente al control (49,1% ± 6,4% vs. 52,6% ± 5,2%).

Conclusiones

Los datos obtenidos sugieren que la tiorredoxina-1 es capaz de reducir el tamaño del infarto en los ratones jóvenes que la sobreexpresan; sin embargo, no se han evidenciado cambios en la función ventricular. Además, el efecto protector del antioxidante se abole en los ratones transgénicos en edad media de la vida.

Palabras clave > Isquemia miocárdica - Reperfusión - Infarto del miocardio - Función ventricular - Antioxidantes - Tiorredoxina

Conflicts of interest
None declared.

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