delay early vascular healing [11], the causes of ST and MI are multifactorial and the precise reasons for these conflict findings remain to be established.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2014.03.167.

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Rosuvastatin increases myocardial microvessels in SHR rats. Role of thioredoxin-1 and peroxiredoxin-2 expression ☆

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Beyond their lipid-lowering action, rosuvastatin possesses pleiotropic effects including anti-inflammatory, pro-angiogenic, antioxidant effects and protective actions against endothelial dysfunction [1,2]. The spontaneously hypertensive rats (SHR) have been extensively studied as a model of essential hypertension. Analogous to that seen in humans, prolonged periods of hypertension produce left ventricular (LV) remodeling, hypertrophy and oxidative stress. Thioredoxin (Trx) and peroxiredoxin (Prx) are a ubiquitous family of cysteine-dependent antioxidant proteins, present in mammalian cells including the heart. Several reports exist in the literature indicating that these proteins are induced by oxidative stress [3]. We investigated the possible effect of long-term monotherapy with rosuvastatin in myocardial expression of redoxins and vascular endothelial growth factor (VEGF), and their relations with LV remodeling in adult SHR. Eight-week-old male SHR and Wistar-Kyoto (WKY) rats, were divided into three groups: SHR (n = 20), SHR-R (n = 20; rosuvastatin 10 mg/kg/day) and WKY (n = 20). For 16 months, animals were housed in individual cages and were given

Table 1
Body weight, left ventricular mass, systolic blood pressure, stereological and immunohistochemical parameters in the experimental groups.

	Groups		
	SHR	SHR-R	WKY
Body weight (g)	438.0 ± 47.2 NS	432.9 ± 27.7 NS	471.3 ± 36.6 NS
LV mass (mg/g)	1470.4 ± 188.6*	1407.8 ± 283.1*	1095 ± 53.1
SBP (mm Hg)	205.3 ± 7.2*	191.5 ± 9.8*	145.5 ± 3.6
Vv_{myo} (%)	76.97 ± 6.645*	74.9 ± 7.8*	83.92 ± 5.769
Vv_{int} (%)	17.8 ± 6.851*	16 ± 6.6*	8.95 ± 4.595
Vv_{ccap} (%)	5.1 ± 2.015*	9.1 ± 2.8**	7.13 ± 2.377
MCSA (μm^2)	1120 ± 419.2*	1125 ± 339.6*	683.5 ± 259.8
CCD (no./mm2)	1493 ± 580.6*	2619 ± 831.4**	2054 ± 685
VEGF score (%)	6.6 ± 4.2	72.1 ± 15.7**	14.77 ± 7.8
Trx1 score (%)	50 ± 24.8*	103.3 ± 36**	72 ± 27.5
Prx2 score (%)	66.6 ± 9.5	135 ± 45.7**	80 ± 23.4

Values are means ± SD. Differences were analyzed using the Kruskal–Wallis test and Dunn's multiple comparison test. Vv_{myo}: volume density of myocardium; Vv_{int}: volume density of interstitium; Vv_{ccap}: volume density of coronary microvessels; MCSA: myocyte mean cross-sectional area; CCD: coronary capillaries density; VEGF: vascular endothelial growth factor; Trx1: thioredoxin 1; Prx2: peroxiredoxin 2.
* $p < 0.05$ when compared with WKY group.
** $p < 0.05$ when compared with SHR and WKY groups.

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[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

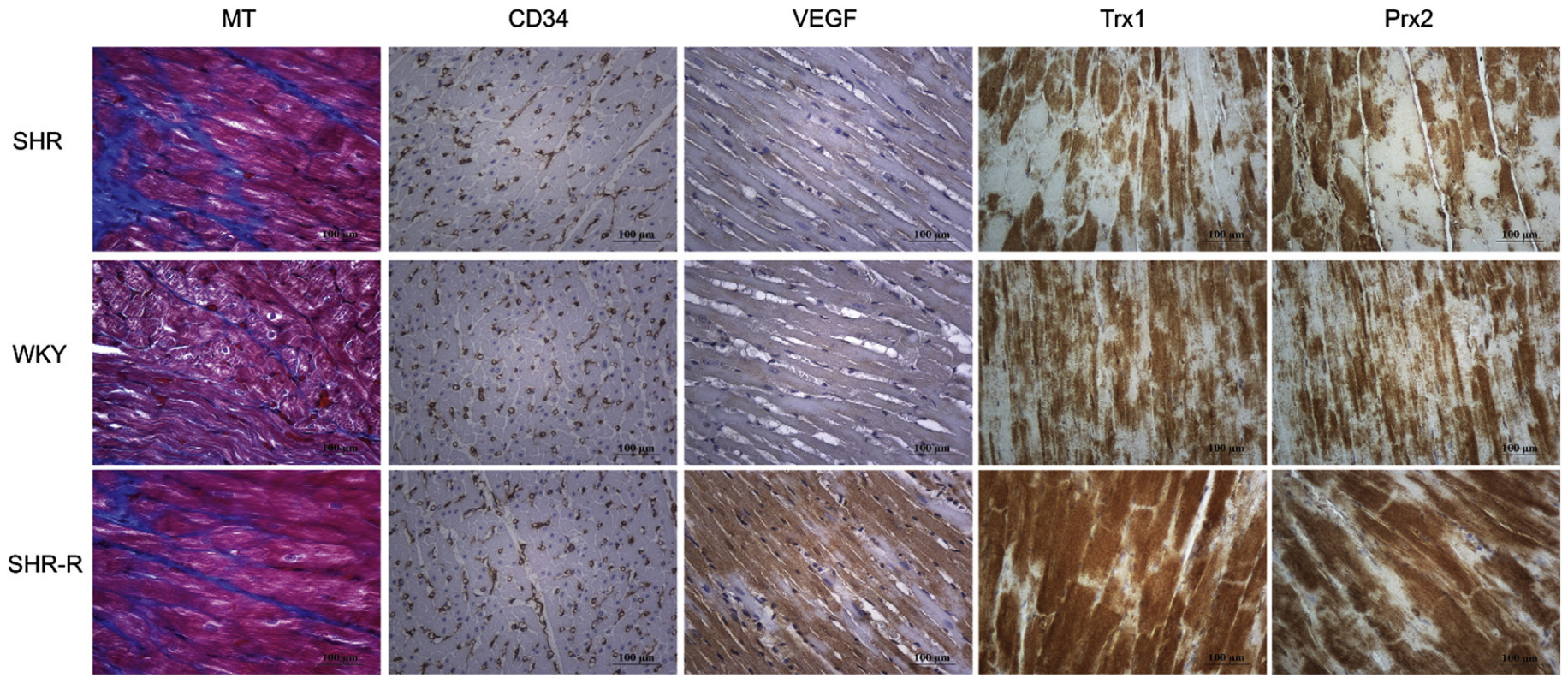

Fig. 1. Representative microphotographs of left ventricle stained with Massons trichrome (MT) from experimental groups. SHR rats showed myocardial hypertrophy and interstitial fibrosis (in blue) as compared to WKY (normotensive) rats. Rosuvastatin therapy not only improved these morphological changes, but also increased the immunohistochemical scoring for CD34, VEGF, Trx1 and Prx2 in the left ventricle. Magnification ×40. Scale bar: 100 µm.

free access to tap water and standard rat chow. At 18 months of age, all the rats were sacrificed and the heart excised and processing for quantitative microscopy and immunohistochemistry studies. Animal care was according to the 'Guide for the Care and Use of Laboratory Animals' published by the US National Institutes of Health (NIH publication no. 85-53, revised 1998). At baseline and after every 2 weeks systolic blood pressure (SBP) was measured by tail-cuff plethysmography. For stereological analysis [4], thin sections from LV tissue blocks were stained with Masson's trichrome, and the volume density (Vv; expressed in percentage) of myocardium (Vv_{myo}), interstitium (Vv_{int}) and coronary capillaries (Vv_{ccap}) were evaluated. In addition, to estimate the individual myocyte mean cross-sectional area (MCSA; expressed in μm^2) and the coronary capillaries density (CCD; expressed as capillary profiles/mm^2 of LV myocardium) an image processing software was used. Immunolabeling of specimens was carried out using a modified avidin–biotin–peroxidase complex technique and expressed as a semiquantitative score [5]. In order to determine the Vv and density of coronary capillaries a polyclonal mouse anti-CD34 primary antibody was used. Myocardial intensity and percentage of VEGF, Trx1 and Prx2 staining were established using the respective primary polyclonal mouse antibody. Control sections were incubated with non-immune normal rabbit serum. Both the number and density of coronary capillaries were significantly increased in SHR-R compared to SHR group. Table 1 summarizes the analyzed stereological and immunohistochemical parameters. Also, the immunohistochemical scoring for VEGF, Trx1 and Prx2 in response to rosuvastatin were significantly higher in myocardium as compared with SHR. However, no significant changes were seen between SHR-R and SHR in stereological parameters such as MCSA, Vv_{myo} and Vv_{int}. Persistently elevated SBP values without changes in LV mass (LV weight normalized to body weight) were observed in SHR-R compared to SHR. Moreover, SHR-R and SHR values were significantly higher in all stereological parameters, SBP and LV mass as compared with WKY. Also, other myocardial immunohistochemical differences were seen between SHR-R, SHR and WKY groups. Our investigation suggests that long-term monotherapy with 10 mg/kg/day of rosuvastatin increases the number and density of LV coronary capillaries and myocardial expression of VEGF in adult SHR, associated to higher immunolabeling for Trx1 and Prx2. (See Fig. 1.)

Cells protect themselves against reactive oxygen species (ROS) through a combination of enzymes and low-molecular-mass antioxidants. In this connection, thioredoxin system appears to play a crucial role in the redox regulation of the ROS signaling, comprising NADPH, thioredoxin reductase (TrxR) and thioredoxin, covering the cytoplasmic Trx1 [6]. The formation of protein and peptide hydroperoxides is of biological significance not only because their generation results in modification of the structure and properties of the amino acid residue, but also because hydroperoxide groups are themselves powerful oxidants. A further group of proteins involved in removing H_2O_2 are the peroxiredoxins that include cytosolic Prx2 [7]. The inhibition of NADPH oxidase is a novel pleiotropic effect described for rosuvastatin [8] that hence could explain our results. This activity may increase NADPH availability to reduce oxidized Trx1 and Prx2, accelerating the cellular antioxidant system, enhanced molecular consumption and inducing redoxin new synthesis. Then, overexpression of Trx1 seems to stimulate VEGF and angiogenesis [9]. Although, long-term therapy with rosuvastatin does not improve LVremodeling nor SBP, our findings suggest antioxidant and pro-angiogenic effects mediated by redoxin systems in adult SHR.

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