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Tempol inhibits TGF-β and MMPs upregulation and prevents cardiac hypertensive changes

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ABSTRACT

Background: Increased oxidative stress upregulates matrix metalloproteinases (MMPs) and transforming grow factor (TGF-β), which are involved in hypertensive cardiac remodeling. We tested the hypothesis that tempol (an antioxidant) could prevent these alterations in two-kidney, one-clip (2K1C) hypertension.
Methods: Sham-operated or hypertensive rats were treated with tempol (18 mg.kg^{-1} day^{-1} or vehicle) for 8 weeks. Systolic blood pressure was monitored weekly. At the end of the treatment, a catheter was inserted into the left carotid artery and into the left ventricle (LV) to assess arterial blood pressure and contractile function. Morphometry of the LV was carried out in hematoxylin/eosin sections and fibrosis was assessed in picrosirius red-stained sections. Cardiac TGF-β level was evaluated by immunofluorescence. Cardiac MMP-2 levels and activity were determined by gelatin zymography, *in situ* zymography, and immunofluorescence. Cardiac superoxide production was evaluated by dihydroethidium probe.
Results: Tempol treatment attenuated 2K1C-induced hypertension and reversed the contractile dysfunction in 2K1C rats. Cardiac hypertrophy was ameliorated by antioxidant treatment. Hypertensive rats showed increased cardiac MMP-2 levels, however tempol did not decrease MP-2 levels. Increased TGF-β level, total gelatinolytic dative stress, ical and functional alterations found in 2K1C-induced cardiac hypertrophy. These findings are consistent with the idea that antioxidants may help to prevent hypertension-induced cardiac hypertrophy.

1. Introduction

Chronic hypertension-induced left ventricular hypertrophy (LVH) usually progresses to a decompensated stage and heart failure [1–3], which may result of altered myocardial collagen matrix and ventricular remodeling [4,5]. Indeed, hypertension-induced progressive accumulation of interstitial collagen fibers may disrupt myocyte alignment and impair cardiac contractile function [4,6]. In this respect, growing evidence indicates that matrix metalloproteinases (MMPs) are involved in the progression of cardiac hypertrophy to heart failure [7,8]. These enzymes degrade fibrillar collagen and contribute to cardiac dysfunction and left ventricle remodeling in hypertension [3,9]. Importantly, MMP-2 overexpression induced severe ventricular remodeling and systolic dysfunction in absence of superimposed injury [10], and this effect may be a result of MMP-2 activity affecting intracellular targets, possibly impairing myocardial contractility [11,12].

Imbalanced MMPs activities result in deposition of extracellular matrix modulated by increased expression of transforming grown factor-β (TGF-β) [13,14], an important mechanism for transition of fibroblasts to myofibroblasts, thus promoting fibrosis [3]. In this regard, increased MMPs and TGF-β levels were shown in LVH [2,3], and both, MMPs and TGF-β, are activated by oxidative stress [14–17]. Therefore, it is possible that antioxidant treatment abrogates cardiac oxidative stress and prevents LVH by mitigating this pathogenic mechanism [3,9,18,19].

Tempol is an antioxidant drug which showed protective effects in many disease models including hypertension [20]. In fact, this promising drug inhibited ROS formation *in vitro* better than other antioxidants [20,21]. In the present study, we hypothesized that the antioxidant effects exerted by tempol would be associated with lower TGF-β levels

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and blunted MMPs activities in the hearts of 2K1C hypertensive rats, which could explain protective effects of tempol against morphological and functional cardiac alterations induced by 2K1C hypertension.

2. Methods

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

2.1. Animals and treatments

Experimental protocols followed standards and policies of the University of Sao Paulo's Animal Care and Use Committee and the animals received humane care. Male Wistar rats (180–200 g) obtained from the colony at University of São Paulo were maintained on 12-h light/dark cycle at 25 °C with free access to rat chow and water.

2K1C hypertension was induced in rats as previously described [22]. Briefly, male Wistar rats (180 g) were anesthetized using ketamine (100 mg/kg intraperitoneally) and xylazine (100 mg/kg intraperitoneally) and, after a midline laparotomy, a silver clip with an internal diameter of 0.20 mm was placed around the left renal artery. Sham-operated rats underwent the same surgical procedure, except for the placement of the renal artery clip. The animals (10 per group) were randomly assigned to 1 of 4 experimental groups as follows: 2K1C and sham groups that received vehicle (water); 2K1C and sham groups that received tempol 18 mg/Kg/day (4-hydroxy-tempo; Sigma; diluted in water) by gavage [23]. Treatment was started 2 weeks after 2K1C hypertension was induced and maintained for additional 8 weeks. Tail systolic blood pressure (SBP) was assessed weekly by tail-cuff plethysmography, and rats were considered to be hypertensive when SBP was higher than 160 mm Hg, 2 weeks after the surgery.

2.2. Assessment of Left ventricular function

Eight rats of each group were anesthetized with tribromoethanol (250 mg/kg, intraperitoneally) after 8 weeks of treatment. Polyethylene catheters (Becton Dickinson and Co, Sparks, MD) were inserted into the right femoral artery for the direct measurement of arterial blood pressure. Another polyethylene catheter was inserted into the right carotid artery, and was carefully introduced into the left ventricle for measurement of left ventricular pressures. The arterial and ventricular catheters were connected to pressure transducers (P23Gb; Statham, Hato Rey, PR) and the signals were properly amplified. Arterial and left ventricular pressures were sampled continuously (1 kHz) on an IBM/PC equipped with an analog to digital interface (DI-220; Dataq Instruments, Akron, OH). A computer program (Advanced CODAS; Dataq Instruments) was used to analyze the data and extract beat-by-beat time series of heart rate (HR) and mean arterial pressure (MAP).

The first derivative of left ventricular pressure (dP/dt) was calculated and values of the maximum rate of isovolumic pressure development (+dP/dtmax) and the maximum rate of isovolumic pressure decay (−dP/dtmax) were measured [24]. At the end of the experiments, the hearts were harvested and washed in saline and the left ventricle was frozen and stored at −80 °C until used for biochemical determinations.

2.3. Harvesting and preparation of the hearts

After 8 weeks of treatment the animals were weighed, anesthetized, and their thoracic cavity was opened to expose the still beating heart. The hearts were rapidly removed, rinsed in ice cold 0.9% saline solution, blotted, weighed, and fixed as a whole in phosphate-buffered 10% formalin (pH 7.3) for histological study. Both ventricles from each heart were isolated and cut into 2 fragments by a mid-ventricular coronal section. Each block was serially cut in the same direction and 4-μm thick sections were stained with hematoxylin and eosin. The left ventricular wall and septum thickness, and the area corresponding to the left ventricular chamber were determined in the first 3 histological sections of each block. Morphometric analyses were carried out with Image J software (http://rsb.info.nih.gov/nih-image/). Average myocytes diameter was determined from 20 measurements in each ventricle, which were made at a magnification of 400× in longitudinally oriented myofibers. Myocyte diameter was obtained in the region of the nucleus of those cells in which the nuclear envelope was sharply defined at both ends, which corresponds to the nucleus length and to its position at an equal distance from the sides of the myocytes. A skilled observer blinded to the treatment groups made the measurements with the Leica Qwin software (Leica Imaging Systems Ltd, Cambridge, UK) in conjunction with a Leica microscope (Leica DMR, Leica Microsystems Wetzlar GmbH, Wetzlar, Germany), videocamera, and an online computer. To estimate the volume fraction (%) of fibrosis in picrosirius red-stained sections, quantitative examination of the left ventricular and septum myocardium was carried out on a medium power light-microscopic field (X400). For each heart, 15 fields per region per rat were randomly selected and analyzed using Leica Qwin software (Leica Imaging Systems). The mean value was subsequently calculated.

2.4. Assessment of cardiac ROS

Dihydroethidium (DHE), a sensitive probe for superoxide (O_2^-) and other non-superoxide ROS, was used to evaluate in situ production of these species. Briefly, cardiac tissues were embedded in Tissue-tek and then frozen and cut in serial 5 μm. Unfixed cryosections were incubated at room temperature, in the dark, with 30 μL of DHE (10 μmol/L) for 30 min. Sections were examined by fluorescence microscopy (Leica imaging Systems Ltd., Cambridge, England) and the image was captured at magnification of 400×. Red fluorescence reflected superoxide and other ROS production and was evaluated by using Image J software.

2.5. Immunofluorescence to assess TGF-beta expression

For immunofluorescence, heart was harvested as above and embedded in paraffin. Immunolabelling was performed with the following primary antibody transforming growth factors beta (polyclonal rabbit anti-transforming growth factor-b (TGF-b)1/2/ 3; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; diluted 1:100]. Sections 5 μm thick were placed on silane coated slides, deparaffinized, washed in PBS, and then submitted to nonspecific antibody binding block. After, the sections were incubated (overnight) with the primary antibody and red fluorescence was visualized by adding a conjugated anti-mouse secondary antibody (Texas red® anti-rabbit, Vector laboratories; 1:200) for 1 h in a dark humidified chamber. The red fluorescence was evaluated using the ImageJ software.

2.6. In situ zymography and immunofluorescence to assess MMP-2 expression

MMPs activities in situ were measured in frozen left ventricles (5 per group) using DQ Gelatin (E12055, Molecular Probes, OR) as a fluorogenic substrate. Briefly, left ventricles samples were embedded in Tissue Tek and cut into 5-μm sections with a cryostat. Sample sections were incubated with 1.0 mg/mL DQ gelatin in Tris–CaCl2 buffer (50 mM Tris, 10 mM CaCl2, 1 mM ZnCl2) in dark humidified chambers for 1 h. The sections were examined with fluorescent microscopy (Leica Imaging Systems Ltd., Cambridge, England) and the image was captured at magnification of 400×. Proteolytic activity was detected as bright green fluorescence, which indicates substrate breakdown, and was evaluated by using ImageJ Program (National Institutes of Health). To evaluate MMP-2 expression, 5-μm tissue sections were incubated with mouse anti-MMP-2 monoclonal antibody (MAB3308, Chemicon, USA,), for 1 h in a dark humidified chamber (at a 1:1000 dilution). Red fluorescence was visualized by adding a rhodamine-conjugated anti-mouse secondary antibody (1:200) (AP160P, Chemicon) for 1 h. To confirm the specificity of antibodies, the primary antibody was omitted and substituted by phosphate-buffered saline 1% BSA. Rhodamine did not bind nonspecifically to the tissue sections. MMP-2 expression was detected as bright red fluorescence, and was evaluated using the ImageJ software.

2.7. Measurement of cardiac MMP-2 levels by gelatin zymography

In the present study, gelatin zymography of MMP-2 and MMP-9 from left ventricle samples was performed as previously described [25]. These MMPs are separated by molecular weight after gel electrophoresis under denaturing conditions. Thereafter, the enzymes are refolded and the proteolytic activity of each form is visualized in the stained zymograms. Briefly, frozen left ventricle samples (10 per group) were homogenized in extraction buffer (300 mL for each 0.1 g of left ventricle sample) containing 10 mM CaCl2, 50 mM Tris–HCl pH 7.4, 0.1% Brij, 0.15 M NaCl, 1 mM Phe (1,10 orto phenanthrolene), 1 mM phenylmethanesulphonylfluoride, and 1 mM N-ethylmaleimide. The samples were placed on ice within a refrigerator for 20 h and then centrifuged at 10,000 *g* for 15 min. The protein content was measured using the Bradford method (Sigma). Thereafter, the samples were diluted 1:1 with sample buffer (final concentration: 2% SDS, 125 mM Tris–HCl; pH 6.8, 10% glycerol, and 0.001% bromophenol blue), and subjected to electrophoresis on 12% SDS-polyacrylamide gel electrophoresis co-polymerized with gelatin (0.1%) as the substrate. After electrophoresis was completed, the gel was incubated for 1 h at room temperature in a 2% Triton X-100 solution, and incubated at 37 °C for 40 h in Tris–HCl buffer, pH 7.4, containing 10 mmol/L CaCl2. The gels were stained with 0.05% Coomassie Brilliant Blue G-250, and then destained with 30% methanol and 10% acetic acid. Gelatinolytic activities were detected as unstained bands against the background of Coomassie blue-stained gelatin. Enzyme activity was assayed by densitometry using a Kodak Electrophoresis Documentation and Analysis System 290 (Kodak, Rochester, NY). Gelatinolytic activities were normalized with regards to an internal standard (fetal bovine serum) to allow integral analysis and comparison. The forms of MMP-2 were identified as bands at75, 72 and 64 KDa.

3. Statistical analysis

The results are expressed as means ± SEM. Between-group comparisons were assessed by 2-way analysis of variance or by 1-way analysis of variance followed by the Newman–Keuls Multiple Comparison Test. A probability value <0.05 was considered significant.

4. Results

4.1. Tempol treatment improves 2K1C-induced hypertension

No differences were found in baseline systolic blood pressure (SBP) among all groups. SBP increased progressively in 2K1C animals after

surgery, achieving 216 ± 8.8 mm Hg at the end of the study period. Tempol treatment attenuated the increase in SBP in 2K1C rats by approximately 33% (Fig. 1A). Similar results were found when invasive arterial blood pressure measurements were considered ($P<0.05$ versus untreated 2K1C group; Fig. 1B, C and D). Tempol treatment did not affect arterial pressure of sham groups throughout the study period. No differences were found in body weight and heart rate among the study groups (data not shown).

4.2. Tempol treatment attenuates 2K1C-induced cardiac dysfunction

Fig. 2 shows maximum ascending and declining rates of left ventricular pressure (+dP/dT max and −dPdT max, respectively). Hypertension increased both +dP/dT and −dP/dT by approximately 34% when compared with the sham group ($P<0.05$). The tempol treatment blunted these changes in +dP/dT and −dP/dT found in 2K1C hypertensive rats ($P<0.05$; Fig. 2).

4.3. Tempol treatment attenuates 2K1C-induced cardiac remodeling

Fig. 3A shows representative photomicrographs of transverse heart sections. We found increased heart weight/body weight ratio in the hypertensive groups compared with the sham groups ($P<0.05$; Fig. 3B), with approximately 30% higher ratios in the untreated 2K1C group compared with the sham group ($P<0.05$; Fig. 3B). Tempol treatment attenuated the increases in this ratio significantly ($P<0.05$ versus untreated 2K1C; Fig. 3B). In parallel with these results, we found increased septum and LV wall thickness (11% and 21%, respectively) in untreated 2K1C rats when compared with sham groups ($P<0.05$). Tempol treatment prevented these increases in hypertensive rats ($P<0.05$ versus untreated 2K1C; Fig. 3A, C and D).

Fig. 4A shows representative photomicrographs of sections from left ventricles, which were used to assess the minor diameter of myocytes. This diameter was 30% greater in untreated hypertensive rats compared with the sham group ($P<0.05$; Fig. 4B). Tempol treatment decreased this parameter by 17% ($P<0.05$; Fig. 4B).

4.4. Tempol treatment attenuates 2K1C-induced cardiac fibrosis

Fig. 5A shows representative photomicrographs of sections from left ventricles, which were used to assess the collagen surface. The myocardial fibrosis was observed in untreated 2K1C rats by an increase in the amount of endomysial and perimysial collagen matrix (1.7 ± 0.13) when compared with the sham group (0.8 ± 0.01; $P<0.05$; Fig. 5B).

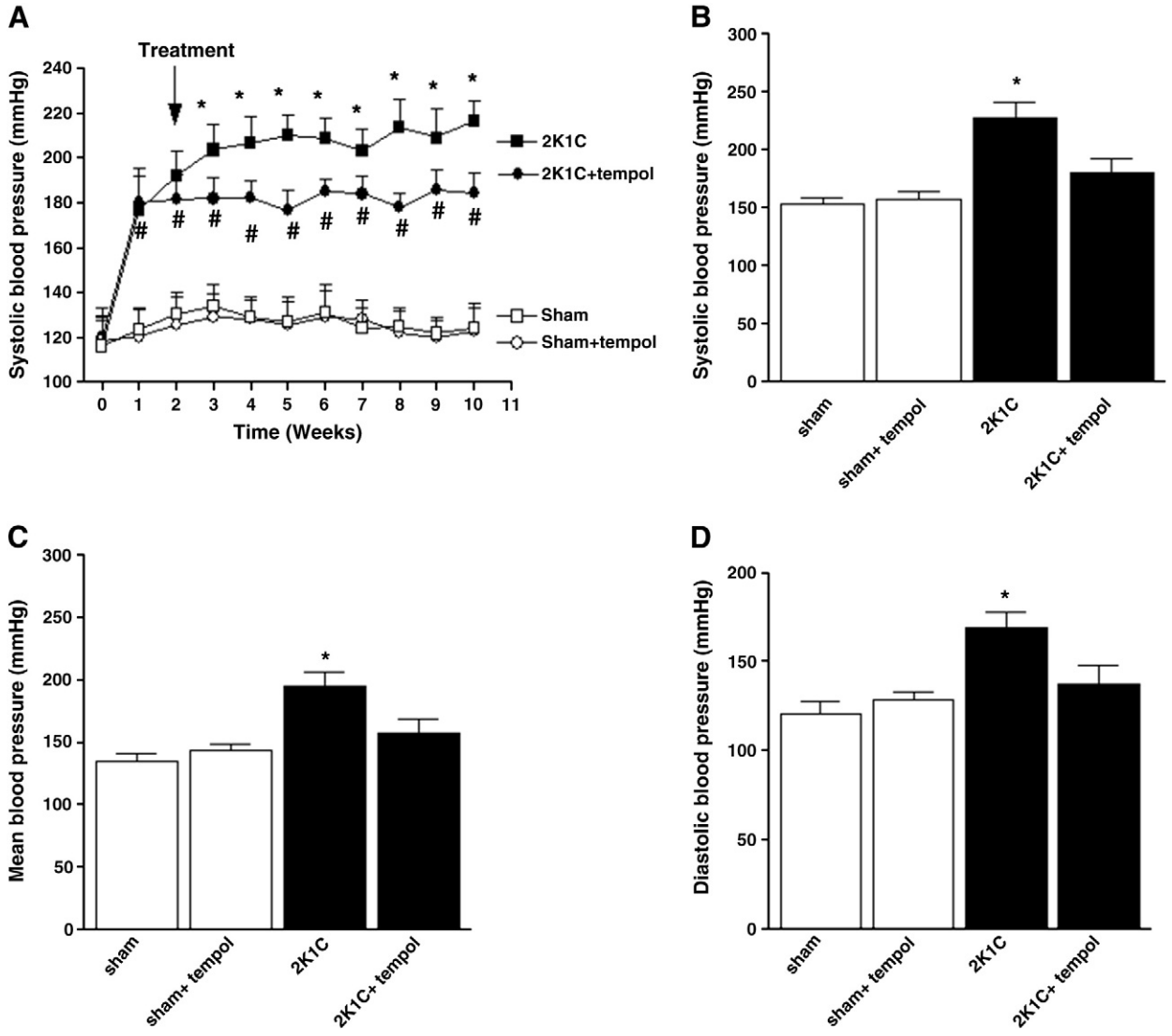

Fig. 1. (A) Systolic blood pressure (mm Hg) measured by tail-cuff method (n = 19–25 per group). (B, C and D) Invasive blood pressure measured with catheters inserted into the right femoral artery. Data are shown as mean ± SEM. (n = 5–9 per group). * $P<0.05$ vs. all the other groups. # $P<0.05$ for 2K1C + tempol group versus the sham groups.

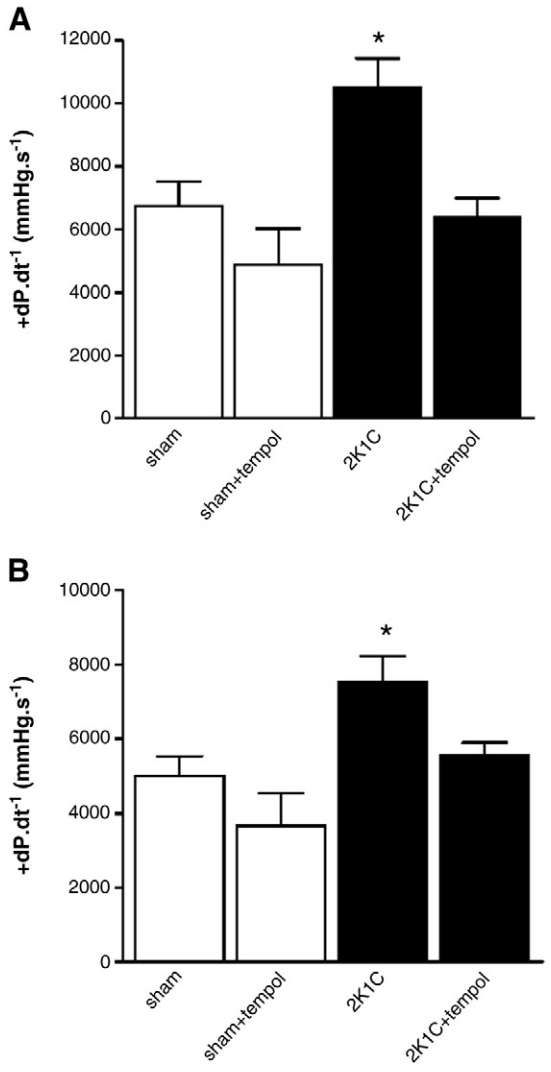

Fig. 2. Increased maximum ascending (panel A; positive dP/dtmax) and declining (panel B; negative dP/dtmax) rates of left ventricular pressure in untreated 2-kidney, 1-clip (2K1C) rats compared with all the other groups. Data are shown as mean ± SEM. (n = 5–8 per group). * $P<0.05$ *vs.* the other groups.

Tempol treatment decreased the myocardial fibrosis in hypertensive rats (1.2 ± 0.07; $P<0.05$; Fig. 5B).

4.5. Tempol treatment inhibits 2K1C hypertension-induced oxidative stress

We evaluated the effects of tempol treatments on ROS levels in the left ventricle of hypertensive rats. Fig. 6A shows representative fluorescence photomicrographs of left ventricular cryosections incubated with DHE. We found higher ROS levels in untreated 2K1C rats compared with the sham group ($P<0.05$; Fig. 6B). Tempol treatment significantly reduced ROS in 2K1C rats when compared to the untreated 2K1C group ($P<0.05$; Fig. 6B).

4.6. Tempol treatment inhibits 2K1C hypertension-induced TGF-beta upregulation

Fig. 7A shows representative photomicrographs of TGF-beta expression. Higher TGF-beta levels were found in LV from 2K1C rats compared with those found in the sham groups ($P<0.05$; Fig. 7B). Treatment with tempol normalized 2K1C induced-cardiac TGF-beta levels upregulation ($P<0.05$; 7B).

4.7. Tempol treatment inhibits 2K1C hypertension-induced MMP activity upregulation, but not MMP-2 expression

Fig. 8A shows representative photomicrographs of total gelatinolytic activity, MMP-2 expression, and their co-localization in the left ventricular tissue. Higher total gelatinolytic activity was found in LV from 2K1C rats compared with those found in the sham group ($P<0.05$; Fig. 8B). Treatment with antioxidant lowered LV gelatinolytic activity ($P<0.05$; 8B). Co-localization of gelatinolytic activity with MMP-2 showed that part of the gelatinolytic activity is probably due to MMP-2 activity.

Increased MMP-2 expression (by immunofluorescence) was found in all hypertensive groups as compared to the sham groups ($P<0.05$; Fig. 8C). Fig. 9A shows a representative SDS-PAGE gelatin zymogram of left ventricle samples. While we found increased MMP-2 levels in the 2K1C group compared with the sham group ($P<0.05$; Fig. 9B), we found no differences among the hypertensive groups ($P>0.05$; Fig. 9B).

5. Discussion

The 2K1C hypertension experimental model is clearly dependent on the activation of the renin–angiotensin–aldosterone system (RAAS), and increased angiotensin II production has direct hypertrophic effects that may be due to increased ROS formation [26]. Indeed, angiotensin II promotes oxidative stress and increases TGF-β [27] levels and MMPs activities [28]. Therefore antioxidant drugs could have important beneficial effects by downregulating these mechanisms.

In the present study, we examined whether antioxidant effects exerted by tempol could prevent the cardiac hypertrophy and upregulation of pro-fibrotic factors (TGF-β and MMPs) in 2K1C hypertensive rats. Our main findings were: (i) antioxidant treatment attenuated the increases in blood pressure, the cardiac hypertrophy and fibrosis found in the 2K1C model, with prevention of cardiac dysfunction; (ii) while tempol did not affect the increases in cardiac MMP-2 levels, it downregulated gelatinolytic activity and TGF-β levels in the left ventricles of hypertensive animals. These findings support the idea that oxidative stress contributes to upregulation of TGF-β levels and MMPs activities in 2K1C hypertension, and that antioxidant treatment may prevent the structural and functional cardiac alterations of hypertension.

Consistent with known cardiac remodeling of extracellular matrix and cellular alterations in hypertensive cardiac disease [2], we found increased cardiac weight paralleling cardiomyocyte hypertrophy and increased collagen deposition in hypertensive animals. These alterations may result from critical involvement of activated RAAS, with increased angiotensin II formation inducing cardiac hypertrophy and fibrosis via TGF-β activation [27]. Moreover, the increased oxidative stress that we found in hypertensive rats may have enhanced TGF-β gene expression and activated latent TGF-β, as previously shown [17]. Conversely, tempol may have blunted this mechanism, as suggested by lower ROS and TGF-β levels in hypertensive rats treated with tempol. These findings are in line with a previous study showing that oxidative stress induces cardiomyocyte hypertrophy and extracellular matrix alterations via TGF-β upregulation in hypertension [14].

The contribution of TGF-β to fibrosis in hypertension involves altered regulation of MMPs activities, as previously shown [2,3,14]. While the precise mechanisms increasing cardiac MMPs activities in 2K1C hypertension are uncertain, we found evidence that increased ROS formation may be a major contributor to imbalanced MMPs activities. In addition, TGF-β may stimulate ROS production by numerous types of nonphagocytic cells including endothelial cells, epithelial cells, smooth muscle cells, and fibroblasts [17],

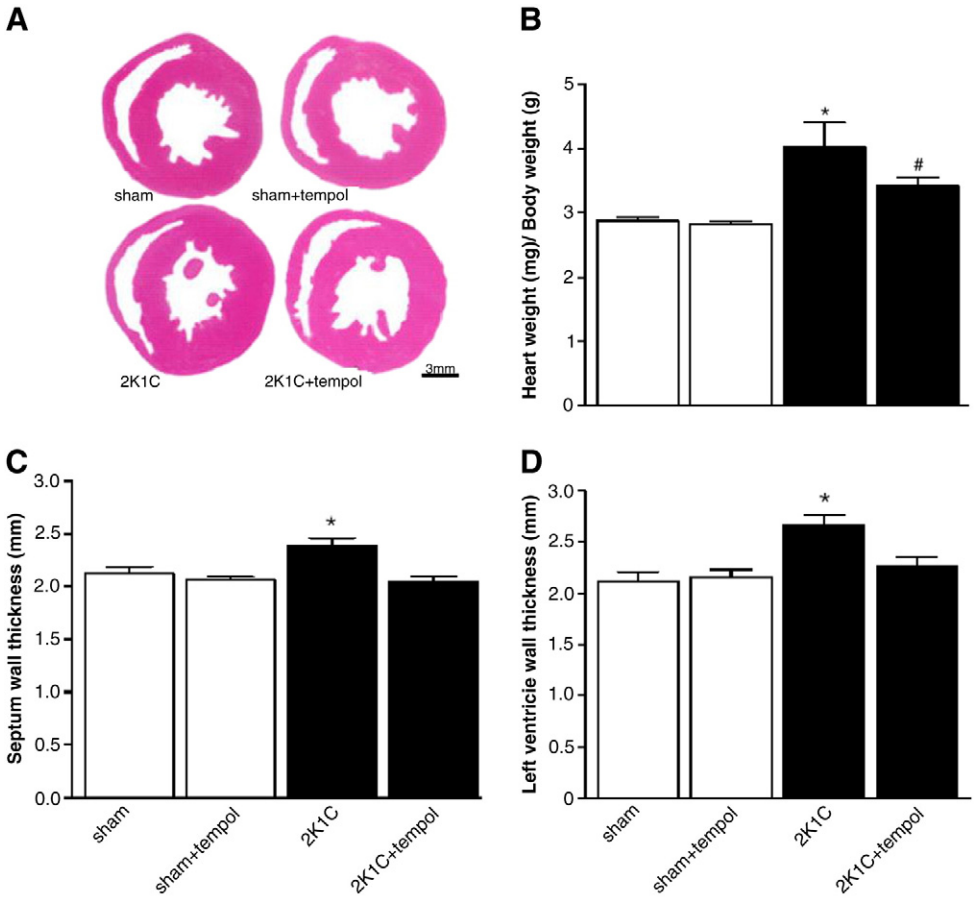

Fig. 3. Effects of tempol treatment on cardiac morphological alterations induced by hypertension. (A) Representative photomicrographs of transverse heart sections used for left ventricle area determination. (B) Body weight/heart weight ratio, (C) septum wall thickness, and (D) left ventricle wall thickness. Data are shown as mean ± SEM. (N = 6 per group). * P<0.05 *vs.* the other groups. # P<0.05 *vs.* the sham groups.

which potentially activate MMPs. In the present study, although tempol had no effects on cardiac MMP-2 levels, it may have blunted MMP-2 activation, since ROS directly activate MMPs, including MMP-2 [15,16,29]. In addition, activation of MMPs by ROS is a multi-stage process, and an initial activation may take place without reduction in the molecular weight of MMPs [30], as it has been described

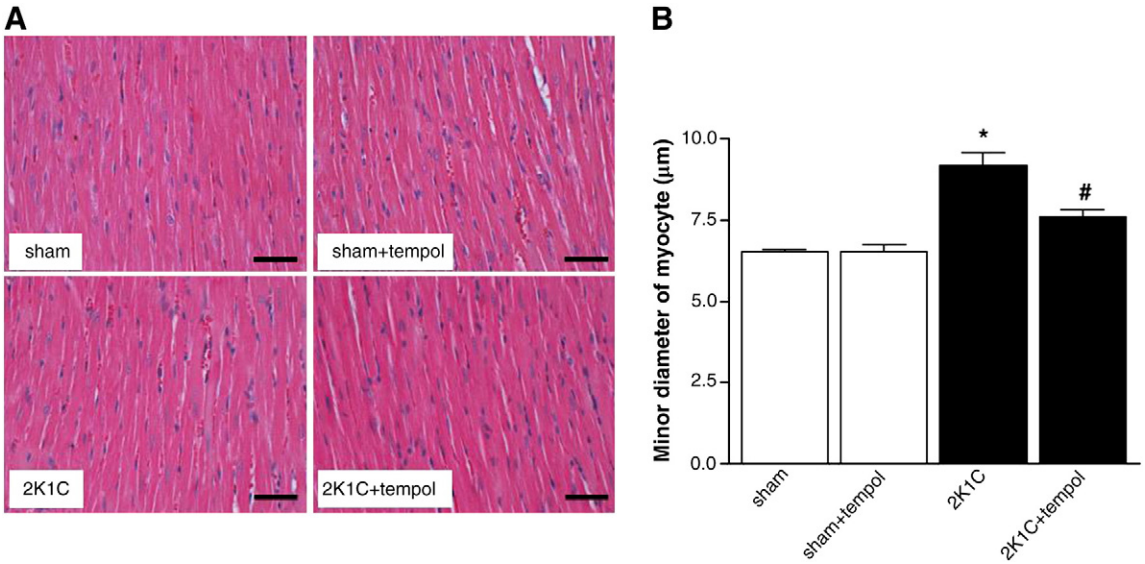

Fig. 4. Antioxidant treatment attenuates hypertension-induced increases in myocyte diameter. (A) Representative photomicrographs of sections from left ventricle myocytes (original magnification 400×). (B) Values of minor diameter of myocytes. Data are shown as mean ± SEM. (N = 4–6 per group). * P<0.05 *vs.* the other groups. # P<0.05 *vs.* the sham groups.

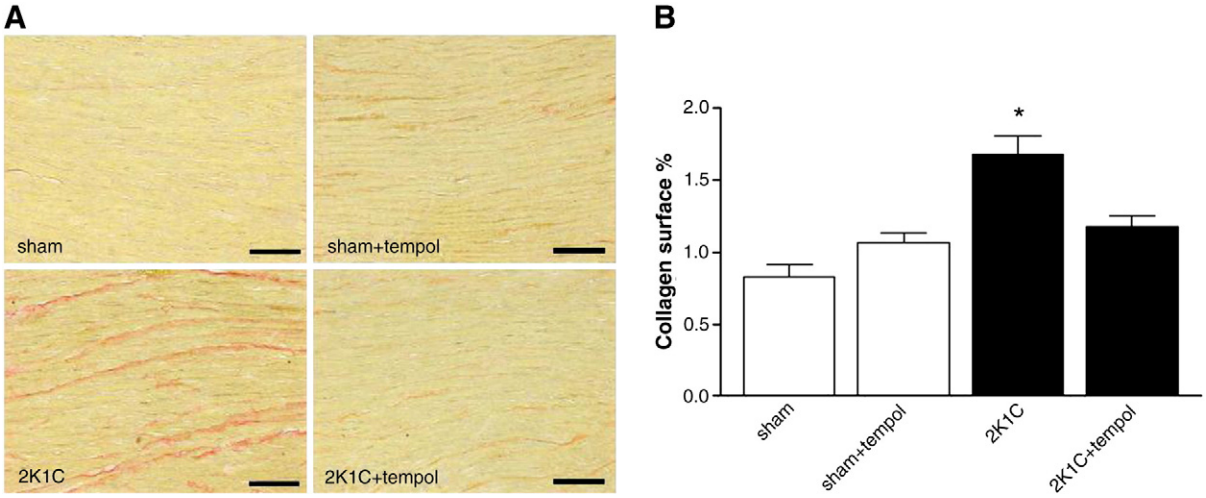

Fig. 5. Antioxidant treatment attenuates hypertension-induced fibrosis. (A) Representative photomicrographs of sections from left ventricle picrosirius red-stained sections (original magnification 400×). (B) Values of volume fraction (%) with fibrosis in the left ventricular and septum myocardium. The mean of 15 fields per region were evaluated for each animal. Data are shown as mean ± SEM. (N = 4–6 per group). * $P<0.05$ *vs.* the other groups.

for MMP-2 [30,31]. Indeed, ROS may interact with thiol groups of the propeptide and with the zinc ion of the catalytic site, thus resulting in activation of the full-length enzyme. Therefore, tempol may have inhibited MMPs activation as a result of its antioxidant effects. Moreover, tempol may have inhibited MMPs activities by promoting the inhibitory properties of the tissue inhibitors of matrix metalloproteinases (TIMPs) in the LV of hypertensive rats. Supporting this suggestion, the ability of TIMPs to inhibit MMPs was lost after treatment with ONOO− or HOCl [32–34]. In a context of impaired TIMP function associated with increased ROS levels, as we found in the hearts of 2K1C rats, the imbalance between MMPs and TIMPs favors increased MMP activity. Therefore, our results strongly suggest that tempol may have downregulated MMPs and exerted protective effects that are similar to those previously found after MMPs inhibition, which attenuated 2K1C-induced functional and morphological cardiac alterations [35].

Other antioxidant drugs may exert similar effects to those exerted by tempol in the present study. Indeed, apocynin attenuated pressure overload-induced cardiac dysfunction, and this effect was associated with reduced cardiac expression of MMPs and TGF-β1 [14], although this has not been shown in the 2K1C model. In fact, this suggestion was apparently valid with respect to the vascular alterations associated with this model [23]. It is also possible that antihypertensive drugs with antioxidant effects may downregulate MMPs and prevent cardiovascular remodeling, as previously suggested [36–40]. However, this suggestion remains to be proved.

In conclusion, our results suggest that tempol protects against 2K1C hypertension-induced structural and functional alterations of the heart. These protective effects are associated with downregulation of TGF-β and MMPs in the heart. Our findings suggest that antioxidants may prevent the activation of key mechanisms involved in hypertensive cardiac hypertrophy.

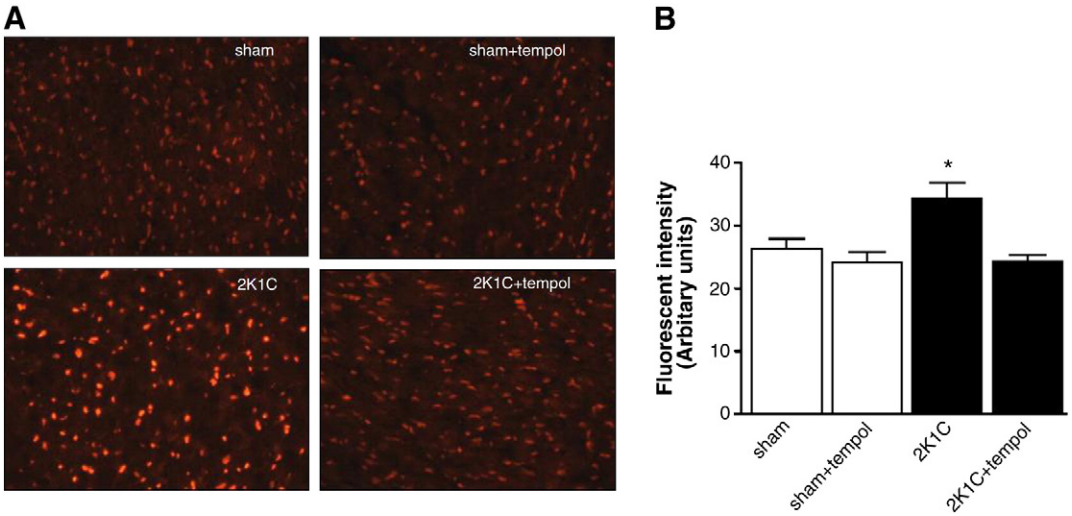

Fig. 6. Effects of antioxidant treatment on cardiac reactive oxygen species production. (A) Representative fluorescence photomicrographs (original magnification 400×) of left ventricular cryosections incubated with DHE. Red fluorescence represents O_2^- production. (B) Quantification of left ventricular fluorescence. Data are shown as mean ± SEM (n = 4–7 per group). * $P<0.05$ *vs.* the other groups.

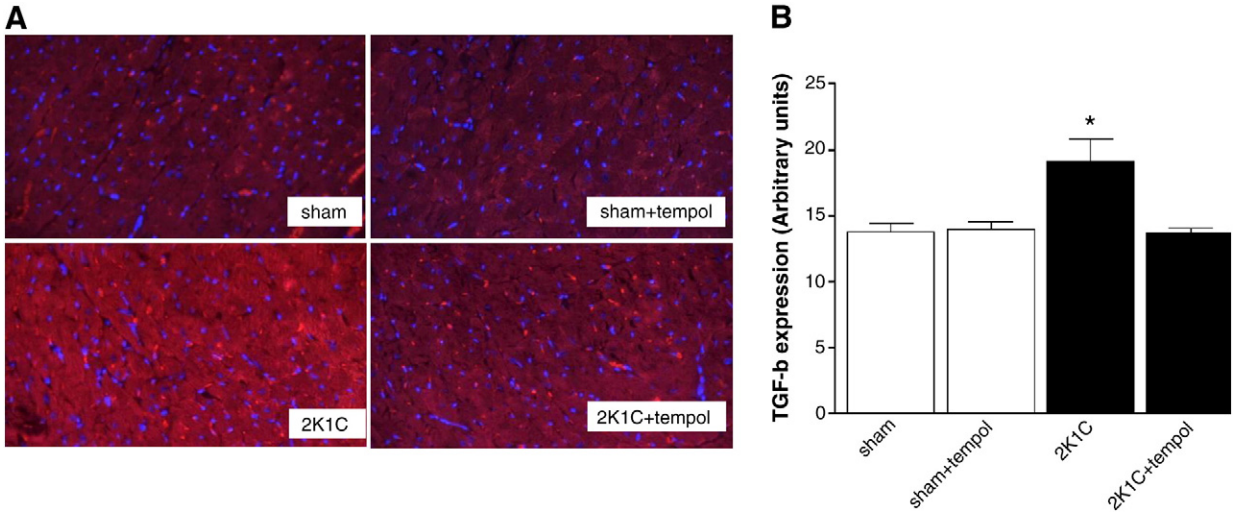

Fig. 7. TGF-β expression in the left ventricles from rats (original magnification 400×). (A) Representative photomicrographs (immunofluorescence) of TGF-β expression. (B) Red fluorescence intensity corresponding to TGF-β levels in the left ventricles from rats. Values are shown as means ± SEM. (n = 4–6 per group). * P < 0.05 *vs.* the other groups.

Acknowledgments

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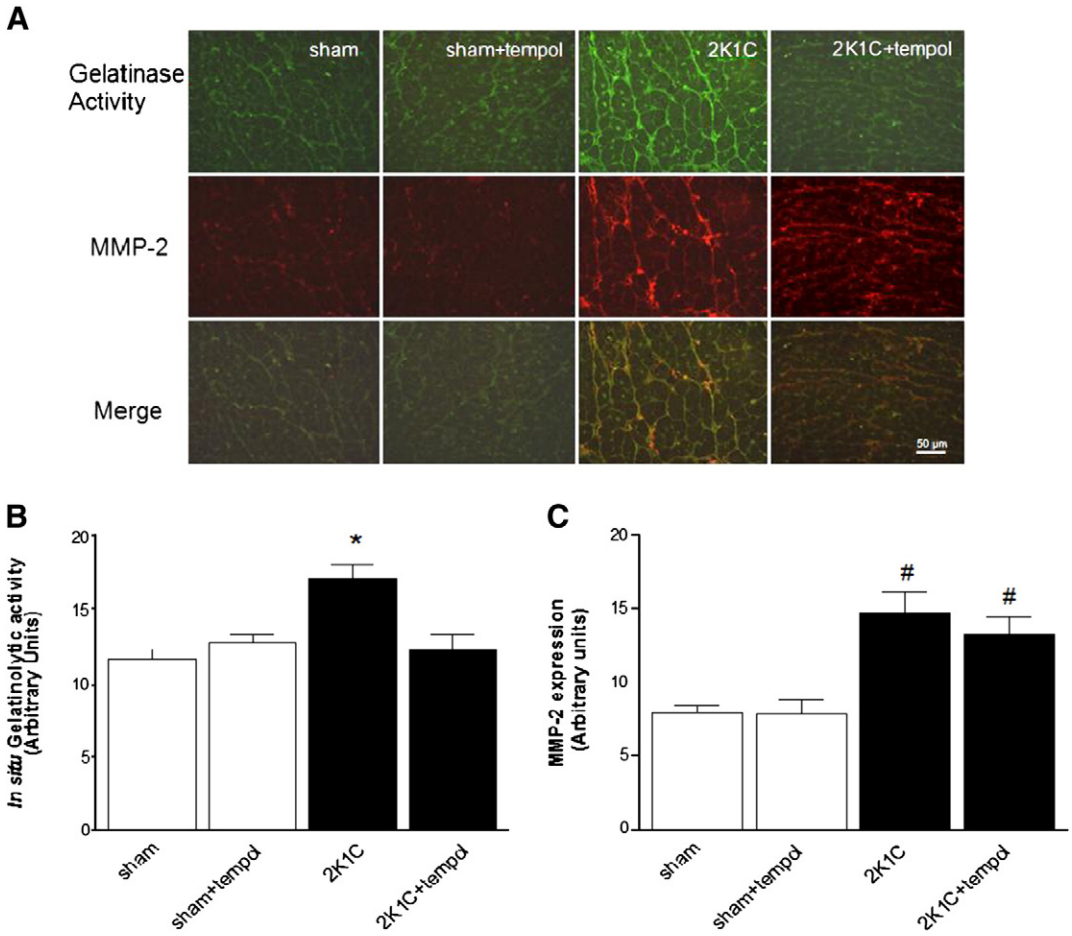

Fig. 8. In situ gelatinolytic activity and MMP-2 expression in the left ventricles from rats. (A) Representative photomicrographs of gelatinolytic activity, MMP-2 staining, and co-localization of both in the left ventricles (original magnification 400×). (B) Quantification of left ventricle surface area covered by bright green fluorescence, which reflects gelatinolytic activity. (C) Red fluorescence intensity, which reflects MMP-2 expression. Values are shown as means ± SEM. (n = 4–6 per group). * P < 0.05 *vs.* the other groups. # P < 0.05 *vs.* the respective sham group.

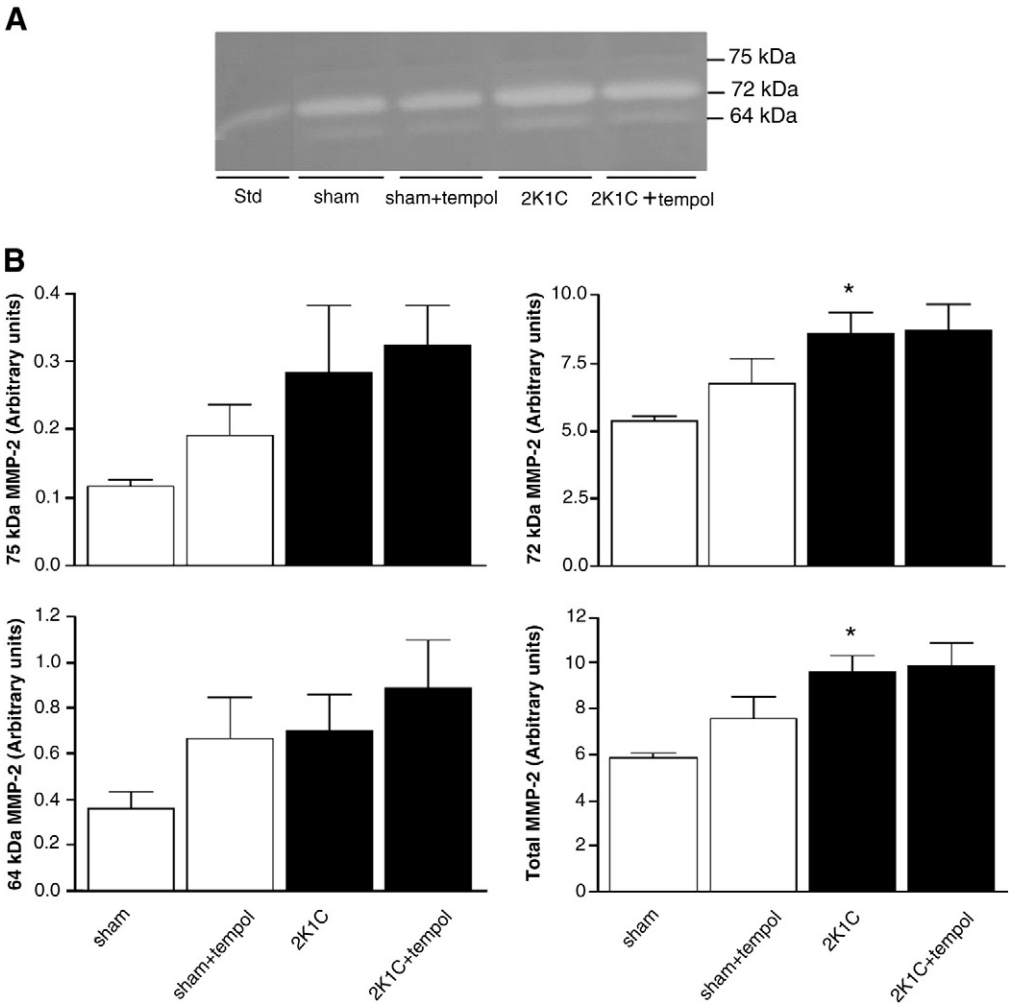

Fig. 9. Representative SDS-PAGE gelatin zymogram of left ventricle samples (A). Std: internal standard. (B) The values for each molecular weight form of MMP-2 (75 kDa, 72 kDa and 64 kDa), and total MMP-2 (75 kDa + 72 kDa + 64 kDa). Data are shown as mean ± SEM. (n = 6 per group). * $P<0.05$ *vs.* sham group.

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