Tempol prevents cardiac oxidative damage and left ventricular dysfunction in the PPAR-α KO mouse

Aziz Guellich,1 Thibaud Damy,2,3,4 Marc Conti,5 Victor Claes,6 Jane-Lise Samuel,7 Thierry Pineau,8 Yves Lecarpentier,9,10 and Catherine Coirault11,12,13,14

1Unité (U)769, Institut National de la Santé et de la Recherche Médicale (INSEMr), Labex-Laboratoire d’Excellence en Recherche sur le Médicament et l’Innovation Thérapeutique, Université Paris Sud, Paris, France; 2Fédération de Cardiologie at the Assistance Publique-Hôpitaux de Paris (AP-HP), Groupe Henri-Mondor Albert-Chenevier, Créteil, France; 3U955, INSERM, Créteil, France; 4Université Paris 12, Faculté de Médecine, Créteil, France; 5Service de Biochimie, Hôpital Henri Mondor, AP-HP, Paris, France; 6Centrum Technologie voor Gehandicapte Personen, University of Antwerp, Antwerp, Belgium; 7Unités Mixtes de Recherche (UMR) S942, INSERM, Université Paris Diderot, Paris, France; 8Département Santé Animale, Institut National de la Recherche Agronomique, Toulouse, France; 9Institut de Cardiologie, Hôpital de la Pitié-Salpêtrière, AP-HP, Universités Paris 6 et Sud, Paris, France; 10Centre de Recherche Clinique, Hôpital de Meaux, Meaux, France; 11U974, INSERM, Paris, France; 12UMR 7215, Centre National de la Recherche Scientifique, Paris, France; 13Université Pierre et Marie Curie, Univ Paris 06 UMR 7676, Paris, France; and 14Institut de Myologie, Paris, France

Submitted 10 September 2012; accepted in final form 28 March 2013

Guellich A, Damy T, Conti M, Claes V, Samuel JL, Pineau T, Lecarpentier Y, Coirault C. Tempol prevents cardiac oxidative damage and left ventricular dysfunction in the PPAR-α KO mouse. Am J Physiol Heart Circ Physiol 304: H1505–H1512, 2013. First published March 29, 2013; doi:10.1152/ajpheart.00669.2012.—Peroxisome proliferator-activated receptor (PPAR)-α deletion induces a profound decrease in MnSOD activity, leading to oxidative stress and left ventricular (LV) dysfunction. We tested the hypothesis that treatment of PPAR-α knockout (KO) mice with the SOD mimetic tempol prevents the heart from pathological remodelling and preserves LV function. Twenty PPAR-α KO mice and 20 age-matched wild-type mice were randomly treated for 8 wk with vehicle or tempol in the drinking water. LV contractile parameters were determined both in vivo using echocardiography and ex vivo using papillary muscle mechanics. Translational and posttranslational modifications of myosin heavy chain protein as well as the expression and activity of major antioxidant enzymes were measured. Tempol treatment did not affect LV function in wild-type mice; however, in PPAR-α KO mice, tempol prevented the decrease in LV ejection fraction and restored the contractile parameters of papillary muscle, including maximum shortening velocity, maximum extent of shortening, and total tension. Moreover, compared with untreated PPAR-α KO mice, myosin heavy chain tyrosine nitration and anion superoxide production were markedly reduced in PPAR-α KO mice after treatment. Tempol also significantly increased glutathione peroxidase and glutathione reductase activities (~50%) in PPAR-α KO mice. In conclusion, these findings demonstrate that treatment with the SOD mimetic tempol can prevent cardiac dysfunction in PPAR-α KO mice by reducing the oxidation of contractile proteins. In addition, we show that the beneficial effects of tempol in PPAR-α KO mice involve activation of the glutathione peroxidase/glutathione reductase system.

oxidative stress; cardiomyopathy; antioxidant therapy; myosin metabolism; peroxisome proliferator-activated receptor-α; knockout

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)-α is a nuclear receptor with pleiotropic effects. In addition to its role in the regulation of lipid metabolism in different tissues, including the heart, PPAR-α plays a central role in the regulation of redox status, i.e., the balance between ROS and their removal by antioxidant systems (22, 32). Both the activation/overexpression or chronic deficiency of PPAR-α results in cardiac oxidative stress, a situation in which cellular antioxidant defences are defective or overwhelmed. PPAR-α deletion leads to a decrease in MnSOD (9), whereas its overexpression results in an increase in H2O2 production through acyl-CoA oxidase in the peroxisome (10). Over the past several decades, clinical and experimental studies have provided evidence demonstrating that oxidative stress is an important contributor to the left ventricular (LV) remodeling responsible for the development and progression of heart failure (HF) (36). Increased production of ROS, especially by mitochondria, has been implicated in a wide range of pathological cardiovascular conditions. ROS can react with basically all cellular compounds, including proteins (31). In muscles such as the heart, contractile proteins and particularly myosin are the major targets, and their oxidative damage can impair contractile performance (6, 12).

Diverse scavenging enzymes, including SOD, glutathione peroxidase (GPx), and catalase, contribute to degrade ROS to less or nontoxic molecules. MnSOD, a key antioxidant enzyme in the heart (21), has the ability to dismutate the superoxide anion into H2O2 (1). MnSOD is regulated by PPAR-α (37), and both MnSOD expression and activity are significantly lower in the hearts of PPAR-α knockout (KO) mice (9).

Pharmacological interventions aimed at the modulation of oxidant-antioxidant homeostasis may represent promising therapies for cardiovascular diseases by preventing protein oxidation. We hypothesized that long-term treatment with the SOD mimetic tempol could reduce oxidative damages in the PPAR-α KO mouse and improve cardiac contractile function.

MATERIALS AND METHODS

Animals. All experimental procedures were performed according to animal welfare regulations, and the study protocol was approved by the Ethical Committee of Paris Diderot University. All investigations conformed with guidelines set by the French Ministry of Agriculture. PPAR-α KO mice were obtained by a targeted deletion of 83 bp in exon 8 of the gene coding for PPAR-α, as previously described (20).
Male PPAR-α KO mice (12 wk old) on a C57BL6 background and their wild-type (WT) littermates were used for this study. Experimental design and treatment. A scheme of the experimental design is shown in Fig. 1. Mice were randomly assigned to four groups. WT and PPAR-α KO mice received either tempol or placebo under the same conditions. In treated groups (WT and PPAR-α KO mice with tempol treatment), tempol (Sigma-Aldrich) was administered at a dose of 1.5 mmol·kg$^{-1}$·day$^{-1}$ in the drinking water. The choice of this dose was based on the results of a previous study (17). All treatments were started at the same age and at the same time. Drink bottles were changed every 2 days in each group. The addition of tempol to the drinking water did not affect water consumption (data not shown).

Blood pressure measurement. Systolic blood pressure (SBP) was measured noninvasively using a computerized tail-cuff system (BP-2000, Visitech Systems, Apex, NC). Mice were held in individual black metal tunnels on a 37°C heated stage. Once mice were in the tunnels, their tails were fitted in an inflatable tail cuff and taped to the infrared base detector platform.

Mice were accustomed to the measurement procedure 4 consecutive days before data collection. The tail-cuff system was calibrated at the beginning of each experiment. Before measurements, mice were left in their metal restraint for a period of 5 min and adapted to the experiment by subjecting them to 10 preliminary inflation-deflation cycles. SBP was then obtained from an average of 10 measurements. Pulse was detected by measuring the change of the blood flow existing between an infrared light source and a photoelectric sensor. The value of SBP was obtained at the disappearance of the wave pulse while the cuff was inflated and recurrence of it during the deflation phase.

Echocardiography. Echocardiography was performed under light isoflurane anesthesia (induction: 2%, maintenance: 0.5–1%, Evaporator Isotec3) using a VIVID 7 machine (Vivid, General Electrics) and a 13-MHz linear probe type M12L (General Electrics). To perform echocardiography measurements at approximately the same heart rate, the isoflurane concentration was adjusted to get a heart rate of ~500 beats/min. During imaging, the mouse was held in the supine position on a 37°C temperature-controlled pad. Interventricular septal thickness, LV posterior wall thickness, and LV internal diameter (LVID) measurements at systole (s) and diastole (d) were determined from short-axis M-mode images obtained at the level of the papillary muscles. Fractional shortening was calculated as follows: (LVIDd − LVIDs)/LVIDd. LV ejection fraction (LVEF) was calculated from the M-mode measurements as follows: (LVIDd$^3$ − LVIDs$^3$)/LVIDd$^3$. Echocardiography was performed in a blind fashion, and offline measurements were averaged from three consecutive cycles.

Papillary muscle mechanics. The anterior LV papillary muscle was prepared as previously described (12). Briefly, mice were anesthetized with pentobarbital sodium (50 mg/kg ip). Hearts were quickly excised and rinsed in a modified Krebs-Henseleit buffer solution with 5% FCS. The solution was bubbled with 95% O$_2$-5% CO$_2$ and maintained at 22°C and pH 7.4. The papillary muscle was mounted on an electromagnetic transducer through one end while the other end was connected to a stationary clip. Transducer technical characteristics and specifications have been previously detailed (12). The papillary muscle was placed in a circulating organ bath and maintained at 22°C and for at least 30 min before the loading protocols were started. Length and tension were collected under electrical stimulation (5-ms stimulation duration, just above threshold) at a frequency of 0.1 Hz, and muscles were stretched to the resting length at which active tension was maximal, i.e., $L_{max}$. Length and tension were recorded from three different contractions: 1) zero-load contraction (the muscle was abruptly clamped from preload to zero load just after the stimulation), 2) isometric contraction (the muscle is loaded with preload only), and 3) isotonic contraction (the muscle was maximal afterloaded and its contraction was isotometric). Mechanical parameters, including the maximal shortening velocity ($V_{max}$, in mm/s), maximum extent of muscle shortening ($\Delta L_{s}$ in %$L_{max}$), and total isometric force, were calculated from contractions 1, 2, and 3, respectively, using self-designed software. Cross-sectional area was measured at the end of each experiment using an optical magnification system. Isometric force was normalized to the cross-sectional area and presented as total tension (TT; in mN/mm$^2$).

Histology. Part of the LV (n = 3–4 hearts/group) was excised and washed in cold PBS. After fixation in 10% buffered formalin, tissues were embedded in paraffin. Sections (5 μm) were stained with hematoxylin-eosin-safran, Sirius red, or Masson’s trichrome. Slides were examined under light microscopy at different magnifications.

Myosin heavy chain electrophoresis. The myosin-enriched fraction was prepared by extracting minced LV biopsies in buffer containing 1 mM EDTA for 30 min at 4°C under magnetic agitation. The suspension was centrifuged at 12,000 g for 10 min. The pellet was subsequently rebuffered in buffer containing 30 mM sodium pyrophosphate buffer (pH 8.8), 1 mM EDTA, 5 mM cysteine, and 50% glycerol. Electrophoresis of myosin heavy chain (MHC) was carried out using myosin extracts were mixed with loading buffer [30 mM Na$_2$P$_2$O$_7$ (pH 8.6), 1 mM EDTA, 5 mM cysteine, and 0.004% bromophenol blue]. The mixture was then denatured at 95°C and loaded onto SDS-8% polyacrylamide gels. Electrophoresis was conducted using a Bio-Rad mini protein II dual slab cell electrophoresis system and was run for 16 h at 4°C and at a voltage of 70 V. Gels were stained with Coomassie brilliant blue R-250, and the relative amounts of fast and slow myosin isoforms (ie, V1 and V3 respectively) in each sample were determined by densitometry using Image Gauge Software.

Western blot analysis. Total protein extracts were obtained from LV tissue. Frozen samples were homogenized in SDS buffer containing 1% SDS, 10 mM Tris-HCl (pH 7.4), and 1 mM Na$_3$VO$_4$ supplemented with protease inhibitors (Complete, Roche Diagnostics). Protein concentrations were determined using the BCA protein assay kit (Pierce). Proteins (10 μg) were resolved by SDS-PAGE (8–12%) and subsequently transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). After being blocked with 5% low-fat milk and Tris-buffered saline for 1 h, membranes were incubated overnight at 4°C with primary antibodies at the appropriate dilution (anti-MnSOD and anti-Cu/ZnSOD antibodies were both from Stressgen and used at 1:1,000, anti-catalase antibody was from Calbiochem and used at 1:1,000, and anti-3-nitrotyrosine antibody was purchased from Santa Cruz Biotechnology and used at a dilution of 1:500). This was followed by a second incubation with the corresponding horse-
radial peroxidase-conjugated secondary antibodies [anti-mouse or anti-rabbit (1:500) for 1 h at room temperature]. Blots were developed using enhanced chemiluminescence substrates (Amersham) to reveal positive bands. Bands were visualized using a highly sensitive imaging system (Fujifilm LAS-3000). Blots were quantified using Image Gauge software and normalized to the expression of actin or myosin.

Antioxidant enzyme activities. Proteins were extracted from frozen tissues in 10 mM phosphate buffer (pH 7.8) and 1 mM EDTA. All measurements were carried out at 37°C. To minimize within-run variations, each sample was measured in duplicate. Activities are expressed in units normalized per milligram of protein. MnSOD activity was measured as previously described (24) using the xanthine-xanthine oxidase system as a generator of superoxide radicals. Quantification was performed at 550 nm. MnSOD activity was measured as previously described (24) using the xanthine-xanthine oxidase system as a generator of superoxide radicals. In brief, red formazan forms when it reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (Randox Laboratories). Total SOD activity was measured at pH 7.8, and CuZnSOD activity was measured at pH 10.2. MnSOD activity was calculated by subtracting CuZnSOD activity from total SOD activity. Catalase activity was measured according to the spectrophotometric method described by Johansson and Borg (15). GPx activity was determined by spectrophotometrically monitoring at 340 nm the oxidation of NADPH in the presence of glutathione reductase (GRase), which catalyzes the reduction of oxidized glutathione (GSSG) formed by GPx using tert-butyl hydroperoxide and H₂O₂ as substrates. The reaction runs at pH 7.5. GRase activity was measured according to the method of Beutler (3). The decrease of reduced NADPH was measured at 340 nm. This index reflects the conversion of GSSG to reduced glutathione (GSH). The reaction was carried out at 37°C.

Glucose-6-phosphate dehydrogenase (G6PDH)/6-phosphogluconate dehydrogenase (6PGD) activity was measured according to Beutler’s method (2). The production of NADPH was measured using 110 μM NADP, 6.6 mM 6-phosphogluconate, and 6.6 mM glucose-6-phosphate.

Markers of oxidative stress. GSSG and GSH were measured by HPLC (19). Samples were purified with 5% metaphosphoric acid. The amount of glutathione within the sample was then measured by HPLC equipped with a colorimetric detection system using N-acetylcyesteine as an internal control.

Direct assessment of the superoxide anion in cardiac tissue was performed using dihydroethidium (DHE; Sigma-Aldrich) staining. LV cryosections were incubated with DHE (37 μM) in a dark humid chamber for 30 min at room temperature. Slides were then washed three times in PBS (3 × 3 min). Three sections from each group were analyzed, and pictures were taken at the same time exposure. The staining intensity was quantified using ImageJ software and expressed in arbitrary units (au) of density. Malondialdehyde (MDA) was assessed by spectrofluorimetry as previously described (7). Briefly, 50-μl samples were incubated for 60 min at 95°C with 1 ml of 10 mM 1,3-diethyl-2-thiobarbituric acid reagent. The thiobarbituric acid-MDA adduct was then extracted by the addition of 5 ml butanol. After centrifugation at 1,500 g for 10 min, the fluorescence of the supernatant was measured at 553 nm after subtraction of the baseline between 500 and 600 nm.

Statistical analysis. Comparisons between groups were assessed by ANOVA. Post hoc analysis was performed using the Fisher test. Differences were considered significant at P values of <0.05.

RESULTS

Animal characteristics and cardiac morphometry. No side effects were reported in the tempol-treated groups. Body weight was measured weekly along the treatment period. Growth curves did not differ between groups, indicating that tempol per se did not significantly affect body weight. The heart-to-body weight ratio did not differ between WT and PPAR-α KO mice. Histogramical analysis of the heart did not reveal morphological differences between the groups (data not shown).

Arterial pressure. SBP was significantly lower in PPAR-α KO mice than in WT mice (104 ± 2 vs. 121 ± 3 mmHg, P < 0.05), as previously reported (12). In WT mice, SBP was significantly lower in the tempol-treated group than in the placebo-treated group (107 ± 4 vs. 121 ± 3 mmHg, P < 0.05). In contrast, tempol-treated PPAR-α KO mice had significant higher SBP compared with untreated PPAR-α KO mice (115 ± 4 vs. 104 ± 2 mmHg, respectively, P < 0.05; Table 1).

Global LV function. At baseline, PPAR-α KO mice exhibited a small but significant lower LVEF compared with WT mice (68 ± 2% vs. 80 ± 3%, P < 0.05; Table 1). Treatment with tempol normalized LVEF in PPAR-α KO mice (P < 0.05 vs. untreated PPAR-α KO mice) but had no significant effect in WT mice (Table 1). LV end-diastolic diameters at diastole were not significantly different between groups, indicating that no dilation occurred (Table 1).

Papillary muscle mechanical performance. Macroscopic aspects of papillary muscles did not differ between groups. In vehicle-treated groups, contractile parameters, including Vmax,
changes in myosin were determined in myosin-enriched fraction samples. 3-Nitrotyrosine immunoreactivity was confined to a weak band in WT groups, whereas a strong band was apparent in samples from untreated PPAR-α KO mice (Fig. 3B). Tempol treatment resulted in an approximately twofold decrease in nitration intensity in PPAR-α KO mice, leading to a level almost similar to that observed in the WT group (Fig. 3B). This indicates that 2-mo treatment with tempol prevented the oxidation of the newly synthesized myosin.

**General markers of oxidative stress.** DHE staining was significantly higher in PPAR-α KO mice compared with WT mice and was significantly reduced after tempol treatment (Fig. 4, A and B). Moreover, in PPAR-α KO mice, tempol also reduced MDA, a product of lipid peroxidation (Fig. 4C). In contrast, tempol did not affect DHE staining or MDA in WT mice. Therefore, our data provided evidence demonstrating that tempol treatment reduced oxidative stress in PPAR-α KO mice.

**Antioxidant enzyme expressions.** The expression of antioxidant enzymes is shown in Fig. 5. In nontreated groups, the expression of antioxidant enzymes was lower in the PPAR-α KO group than in the WT group (Fig. 5A). Treatment with tempol normalized the expression of antioxidant enzymes (Fig. 5B). Therefore, our data provided evidence demonstrating that tempol treatment reduced oxidative stress in PPAR-α KO mice.
PPAR-α KO mice showed a significant lower expression of MnSOD compared with WT mice (2.4 ± 0.19 vs. 3.5 ± 0.41 au, P < 0.05; Fig. 5A). Interestingly, treatment with tempol resulted in a downregulation of MnSOD protein expression in WT mice (2.3 ± 0.17 au, P < 0.05 vs. vehicle) and had no significant effect in PPAR-α KO mice (2.9 ± 0.35 au, P > 0.05 vs. vehicle; Fig. 5A). Expression of CuZnSOD (Fig. 5C) and catalase (Fig. 5B) did not significantly differ between WT and PPAR-α KO mice and was not affected by treatment.

Antioxidant enzyme activities. To determine whether the reduced MnSOD expression in tempol-treated WT mice was associated with reduced enzyme activity, MnSOD and CuZnSOD activities were measured. There were no differences between untreated and treated WT groups with regard to MnSOD (3.07 ± 0.37 vs. 3.48 ± 0.32 U/mg) and CuZnSOD (1.39 ± 0.11 vs. 1.64 ± 0.13) activities. The antioxidant enzyme activities of catalase, GPx, and GRase are shown in Fig. 6. There were no differences in catalase activity between groups (Fig. 6A). GPx activity was similar in nontreated WT and PPAR-α KO mice (Fig. 6B); however, its activity increased...
served in WT mice after treatment (Fig. 6). GSH and GSSG levels were similar in all groups, including the PPAR-α KO group (GSSG: 13.8 ± 1.4, 15.7 ± 2.1, 15.7 ± 2.0, and 13.8 ± 1.4 in untreated WT mice, untreated PPAR-α KO mice, tempol-treated WT mice, and tempol-treated PPAR-α KO mice, respectively; GSH: 86.5 ± 5.9, 88.9 ± 10.7, 89.9 ± 6.3, and 88.8 ± 12.4 in untreated WT mice, untreated PPAR-α KO mice, tempol-treated WT mice, and tempol-treated PPAR-α KO mice, respectively). As a consequence, the GSH-to-GSSG ratio did not differ between groups (data not shown). This was not surprising given that both GPx and GRase were upregulated in treated PPAR-α KO animals. To further investigate how the enzymes involved in the regeneration of the GSH pool were affected, we measured the combined activity of G6PD and 6GPD, the two enzymes that produces NADPH necessary for the regeneration of GSH by GRase (Fig. 6D). Interestingly, 6GPD/6GPD activity was higher in PPAR-α KO mice treated with tempol compared with the other groups (P < 0.05). Taken together, these data suggest that tempol’s mechanism of action involves glutathione metabolism enzymes, which were upregulated in a highly coordinated manner.

**DISCUSSION**

Oxidative stress plays an important role in the initiation and progression of the cardiac structural and functional abnormalities observed in HF. Several studies have shown that oxidative stress induces severe damage on all cellular and tissue compounds (11). Reestablishing the oxidant/antioxidant balance is considered as one of the promising therapeutic strategies to treat HF and several other diseases (1). ROS scavengers were able to preserve structural integrity and LV function in many experimental HF models induced by different stimuli, such as pressure overload (8, 35) or infarction (28). Furthermore, different drugs currently used to treat HF improve cardiac function, at least partially, through their antioxidant effects (4).

In the present study, we used the well-characterized model of the PPAR-α KO mouse to test the hypothesis that a targeted antioxidant therapy reduces ROS-mediated cardiomyopathy. We (12) have previously reported that PPAR-α KO deletion results in an oxidative stress status. Using combined functional and biochemical tests, we proposed a scheme of the origin of the oxidative stress, the target proteins, and the functional consequences. In this scheme, MnSOD expression and activity are significantly lower in PPAR-α KO animals (30% and 50%, respectively). The decrease in MnSOD expression is mainly due to transcriptional regulation, as supported by the presence of a peroxisome proliferator-responsive element sequence on the promoter of MnSOD (37). The reduced MnSOD could be due, at least in part, to the reduced fatty acid oxidation in PPAR-α KO mice. MnSOD is one of the major cardiac antioxidant enzymes and accounts for 70% of the cellular dismutation of the superoxide anion (21). Li et al. (21) demonstrated that MnSOD KO mice have diminished defence against oxidative stress. In parallel, MnSOD overexpression protects against different stresses, including ischemia-reperfusion (5, 14) and diabetes (33).

Potential mechanisms by which reduced mitochondrial MnSOD induced myofilament oxidation need to be discussed. Both the superoxide anion and nitric oxide have a short half-life. However, mitochondria, which occupy ~30% of the cardiomyocyte...
volume (31), are tightly and physically connected to myofilaments (16). This close connection makes contractile proteins a potential target of peroxynitrite formed secondary to superoxide anion externalization (8).

In the present study, we showed that MnSOD is downregulated even in young adult PPAR-α KO mice (3–4 mo), with a subsequent increase in oxidative/nitrosative damage and LV contractile dysfunction even in the absence of hypertrophy and cellular disorganization. In contrast, 9-mo-old PPAR-α KO mice exhibit myofibril disorganization and cardiomyocyte hypertrophy. Combining our previous results with those of the present study gives insights about the origin and time progression of PPAR-α KO-related cardiomyopathy and confirms that the oxidative stress precedes LV hypertrophy and tissue damages.

Native SOD does not cross biological membranes and causes serious immunological reactions, making its use challenging. Different MnSOD mimetics, including tempol, are used as an alternative strategy. Tempol is a well-characterized superoxide anion scavenger (27, 30) that permeates cell membranes. In addition, it is metal independent and does not produce secondary superoxide anions. In the present study, we sought to prevent structural abnormalities and LV dysfunction by administrating tempol at an early age of the disease, i.e., before cardiac hypertrophy or other irreversible damages had occurred. To achieve this goal, we took into consideration the turnover of myosin. We set up the treatment period for 8 wk, which corresponds to 1.5 times the half-life of myosin (34). Interestingly, PPAR-α KO mice treated with tempol exhibited reduced oxidative/nitrosative damage of myosin compared with their vehicle-treated counterparts and preserved global cardiac function, as assessed by echocardiography. Intrinsic contractile LV performance was determined ex vivo in controlled loading conditions so as to exclude the effect of SBP variations between groups and any other systemic or neuroendocrine changes that may influence the global apparent function. $V_{\text{max}}$, $\Delta L$, and TT were lower in vehicle-treated PPAR-α KO mice. Treatment with tempol preserved all these parameters in PPAR-α KO mice, resulting in values similar to those of WT mice. Consistently, in a rat model of drug-induced cardiac toxicity, Lord et al. (23) previously reported a beneficial effect of tempol on cardiac function as well as in the oxidative damage of myofilament proteins, including desmin and myosin light chain. Taken together, these data suggest that the action of tempol is not specific to PPAR-α KO mice. The beneficial effect of tempol on cardiac contractility may also involve other mechanisms, such as an improved ATP supply (as ROS can also reduce mitochondrial function and ATP generation) and/or improved Ca$^{2+}$ homeostasis through the reduction of oxidation of Ca$^{2+}$ cycling proteins such as sarco-(endo)plasmic reticulum Ca$^{2+}$ ATPase.

It has been previously reported that tempol decreases arterial pressure in hypertensive rats (25, 39). Different mechanisms underlying the hypotensive effect of tempol have been proposed: an increase in nitric oxide bioavailability by preventing its interaction with the superoxide anion in the spontaneously hypertensive rat (13) and a direct vasodilator effect or a direct decrease in neuronal activity in the paraventricular nucleus of the hypothalamus and rostral ventrolateral medulla in rats (38). Surprisingly, even with a decrease in MnSOD expression and activity, SBP was significantly lower in PPAR-α KO mice. This could be due to a systemic adaptation to preserve normal cardiac output in young PPAR-α KO mice. We should note that heart rate did not vary according to the genotype (12). More surprisingly, tempol treatment increased SBP in PPAR-α KO mice. The necessary experiments to understand these observations and the link among PPAR-α, tempol, and SBP have yet to be performed.

Enzymes involved in the regulation of the cellular redox balance are usually organized in a multichain enzyme reaction, and they are tightly regulated at the transcriptional level (9). It is also known that a reasonable amount of ROS is necessary for several physiological processes and is required for optimal muscle contraction (29). The reduced MnSOD expression observed in treated WT mice could be explained by a downregulation of the enzyme to maintain a normal superoxide anion scavenging capacity in the mitochondria fulfilled by both MnSOD and tempol. Other possible explanation was that tempol reduced the amount of nitrated MnSOD in WT mice below its basal level (18), leading to an adaptative decrease in its expression (26). Although it has been used in vivo in different studies, few data are available on 1) the mechanism(s) of action of tempol (if its action is direct transformation of the superoxide anion to H$_2$O$_2$) and 2) how tempol is cleared from the cell and what becomes of its byproducts. Pharmacological and toxicological investigations, such as dose-response studies, would bring more insights on the efficacy of tempol. Interestingly, and whatever are the mechanisms, our study showed a significant increase in both GPx and GRase on the one hand and an increase in G6PD and 6GPD on the other hand, suggesting coordinated activation of this system. Taken together, our data highlight the plastic tuning nature of the enzymatic antioxidant system. However, because no efficient anti-GPx and anti-GRase antibodies are commercially available, our study could not ascertain how tempol regulates the signaling pathways. Further studies are required to evaluate the relation between tempol and antioxidant enzymes gene transcription.

In summary, the present study demonstrates that treatment with tempol normalizes global cardiac function and the intrinsic contractile performance of the LV in PPAR-α KO mouse cardiomyopathy. This was associated with a significant decrease in the amount of oxidized myosin. The compound appears to be safe and well tolerated, which makes it a good candidate as an additional treatment for HF.

ACKNOWLEDGMENTS

The authors are thankful to Isabelle Nelson and Jean-Michel Thoridnet for helpful discussions.

GRANTS

This work was supported by grants from Institut National de la Santé et de la Recherche Médicale and the Fédération Française de Cardiologie France. A. Guellich was supported by a grant from the French Ministry of Education, Research and Technology.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


