Antihypertensive effect of mitochondria-targeted proxyl nitroxides

Anna E. Dikalova a, Igor A. Kirilyuk b,c, Sergey I. Dikalov a,∗

a Vanderbilt University Medical Center, 2200 Pierce Avenue, Nashville, TN 37232, USA
b Novosibirsk Institute of Organic Chemistry, Lavrentieva 9, Novosibirsk 630090, Russia
c Novosibirsk State University, Pirogova 2, Novosibirsk 630090, Russia

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A B S T R A C T

Superoxide (O2•−) has been implicated in the pathogenesis of many human diseases including hypertension. Mitochondria-targeted superoxide scavenger mitoTEMPO reduces blood pressure; however, the structure-functional relationships in antihypertensive effect of mitochondria-targeted nitroxides remain unclear. The nitroxides are known to undergo bioreduction into hydroxylamine derivatives which react with O2•− with much lower rate. The nitroxides of pyrrolidine series (proxys) are much more resistant to bioreduction compared to TEMPOL derivatives suggesting that mitochondria-targeted proxys can be effective antioxidants with antihypertensive activity. In this work we have designed and studied two new pyrrolidine mitochondria targeted nitroxides: 3-[2-(triphenylphosphonio)acetamido]- and 3-[2-(triphenylphosphonio)acetamidomethyl]-2,5,5-tetramethylpyrrolidine-1-oxyl (mCP1) and (mCP2). These new mitochondria targeted nitroxides have 3–7-fold lower rate constants of the reaction with O2•− compared with mitoTEMPO; however, the cellular bioreduction of mCP1 and mCP2 was 3– and 2-fold slower. As a consequence incubation with cells afforded much higher intracellular concentration of mCP1 and mCP2 nitroxides compared to mitoTEMPO nitroxide. This has compensated for the difference in the rate of O2•− scavenging and all nitroxides similarly protected mitochondrial respiration in H2O2 treated endothelial cells. Treatment of hypertensive mice with mCP1 and mCP2 (1.4 mg/kg/day) after onset of angiotensin II-induced hypertension significantly reduced blood pressure to 133 ± 5 mmHg and 129 ± 6 mmHg compared to 163 ± 5 mmHg in mice infused with angiotensin II alone. mCP1 and mCP2 reduced vascular O2•− and prevented decrease of endothelial nitric oxide production. These data indicate that resistance to bioreduction play significant role in antioxidant activity of nitroxides. Studies of nitroxide analogs such as mCP1 and mCP2 may help in optimization of chemical structure of mitochondria-targeted nitroxides for improved efficacy and pharmacokinetics of these drugs in treatment of hypertension and many other conditions including atherosclerosis, diabetes and degenerative neurological disorders in which mitochondrial oxidative stress seems to play a role.

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Introduction

Clinical data show that 26% of adult population has hypertension [1]. This disease represents a major risk factor for stroke, myocardial infarction, and heart failure [2]. Hypertension is a multifactorial disorder involving perturbations of the vasculature, the kidney and the central nervous system [3]. Despite treatment with multiple drugs, 37% of hypertensive patients remain hypertensive [4], likely due to the mechanisms contributing to blood pressure elevation that are not affected by current treatments. New classes of antihypertensive agents could therefore add to the currently available therapeutic armamentarium to improve treatment of hypertension.

In the past decade it has become clear that vascular superoxide (O2•−) production contributes to hypertension [5]. In almost all experimental models of hypertension O2•− production is increased in multiple organs, including vasculature where O2•− promote vasoconstriction and remodeling, increasing systemic vascular resistance. In the past several years, we have shown that the mitochondria become dysfunctional in hypertension and have defined novel role of mitochondrial O2•− in this disease [6,7]. The mitochondria are an important source of O2•− and we have shown that scavenging mitochondrial O2•− improves endothelial function and attenuates hypertension [6].

Common antioxidants like ascorbate and vitamin E have proven ineffective in preventing cardiovascular diseases and hypertension [8]. These agents unlikely reach important sites of ROS production such as the mitochondria. Experimental studies have shown an important role of mitochondrial reactive oxygen species in the development of endothelial dysfunction, hypertension and...
atherosclerosis [9,10]. Indeed, we have shown that SOD2 overexpression attenuates hypertension and treatment of hypertensive mice with mitochondria-targeted antioxidants reduces blood pressure [6,7].

The membrane potential of mitochondria within living cells is negative inside (−140 mV). As this membrane potential is far larger than in other organelles within cells, lipophilic cations such as triphenylphosphonium (TPP) selectively accumulate within mitochondria [11]. Antioxidants conjugated to TPP, therefore can be targeted to mitochondria and may be concentrated in the mitochondrial matrix by 1000-fold [12]. The pharmacology of mitochondria-targeted antioxidants is not well understood. For example, previously described mitoquinone (MitoQ10) [12] may have prooxidant and proapoptotic properties due to redox cycling and generation of O$_2^−$ by quinone [13,14]. Nitroxides are well-known generation of prooxidant and proapoptotic properties due to redox cycling and the presence of angiotensin II [6].

Respiration in control cells but improved mitochondrial function in triphephenylmethylphosphonium conjugates should be used at submicromolar concentrations and tested for side effect on respiration. Indeed, low doses of mitoTEMPO did not affect mitochondrial respiration in control cells but improved mitochondrial function in the presence of angiotensin II [6].

Recently, it has been shown that pretreatment of endothelial cells with the mitochondria-targeted SOD mimetic Mito-CP significantly reduces H$_2$O$_2$- and lipid peroxide-induced cellular oxidative stress [19]. Mito-CP inhibits peroxide-induced inactivation of complex I and aconitate, while restoring the mitochondrial membrane potential. In contrast, the “untargeted” carboxy proxyl (CP) did not protect the cells from peroxide-induced oxidative stress and apoptosis. However, in Mito-CP nitroxide is connected to triphenylmethylphosphonium cation via ester spacer and ester group can be potentially hydrolyzed to give inactive 3-carboxypyroxyl (CP).

The main disadvantage of nitroxides is their rapid reduction with cellular antioxidants and enzymatic systems. For example, mitoTEMPO is readily reduced in mitochondria to corresponding hydroxylamine [20]. Hydroxylamine derivatives react with O$_2^−$ much slower compared with nitroxides [6] therefore bioreduction of nitroxides can significantly reduce antioxidant potentials of nitroxides.

We have hypothesized that increased resistance to bioreduction will be beneficial to mitochondria-targeted nitroxides. The structure-functional relationships in antioxidant properties of mitochondria-targeted nitroxides however remain unclear. In this work we have designed and studied two new pyrrolidine mitochondria targeted nitroxides mCP1 and mCP2 (Fig. 1), and studied their antioxidant properties, cellular accumulation, their bioreduction and antihypertensive properties.

### Materials and methods

**Reagents**

Xanthine oxidase was purchased from Roche Molecular Biochemicals (Indianapolis, IN). All other reagents were obtained from Sigma (St Louis, MO).

**Synthesis of mCP1 and mCP2**

3-Amino-PROXYL (1a) and 3-aminomethyl-PROXYL (1b) were prepared according to the literature methods described by Rozantsvev and Hankowsky [22,23]. The nitroxides mCP1 and mCP2 were synthesized according to Fig. 2 similarly to the procedure previously described for mitoTEMPO (mT) [24].

3-(2-Chloroacetamido)methyl)-2,2,5,5-tetramethylpyrroldin-1-oxyl (2b). A solution of 3-aminomethyl-PROXYL (0.9 g or 0.005 mol) and dry triethylamine (1.7 ml or 0.0117 mol) in dry chloroform (20 ml) was placed into 50 ml flat-bottom flask and cooled to −5 °C. The resulting solution was placed into ice bath and chloroacetyl chloride (0.42 ml or 0.005 mol) was added dropwise upon stirring. The resulting dark solution was washed with water (3 × 5 ml), and dried with MgSO$_4$. The chloroform was removed under reduced pressure and the residue was separated by column chromatography (Kieselgel 60, Merck, eluent chloroform) to give 2b. Yellow oil, Found: C, 53.06; H, 8.08; N, 11.56; Cl, 14.10. Calculated for C$_7$H$_7$ClO$_2$: C, 52.99; H, 7.99; N, 11.53; Cl, 14.05; ν$_{max}$ (KBr)/cm$^{-1}$ 3306 br, 3084 br, 2972, 2934, 2872, 1668, 1543, 1462, 1364, 1313, 1252, 1178, 1159, 1107, 1057, 789, 764, 691 br, 600, 571, 525, 476.

Similarly, 3-(2-chloroacetamido)-2,2,5,5-tetramethylpyrroldin-1-oxyl (2a) have been prepared from 3-aminomethyl-PROXYL (1a), (this compound was first described in [Mao-Man-Jun; Tian, Xuan; Chen, Yao-Zu; Gaodeng Xuexiao Huaxue Xuebao (1998), 19(3), 395–398.], but this publication is not available to authors): yellow crystals, m.p. 130–131°C (hexane-ethyl acetate), Found: C, 51.31; H, 7.66; N, 11.87; Cl 15.10. Calculated for C$_7$H$_7$N$_2$O$_2$: C, 51.30; H, 7.76; N, 11.99; Cl 15.17%; ν$_{max}$ (KBr)/cm$^{-1}$ 3319, 3076 br, 2982, 2938, 2876, 1676, 1553, 1485, 1408, 1366, 1331, 1299, 1229, 1196, 1165, 1111, 1045, 806, 772, 687 br, 572, 555, 547, 530, 500.

3-(2-Triphenylphosphinolioacetamido)methyl)-2,2,5,5-tetramethylpyrraldin-1-oxyl (mCP1). The 2b (1 g, 0.004 mol) was placed into 50 ml flask containing toluene (15 ml) and tripheryl phosphine (2.7 g, 0.01 mol). The mixture was heated to reflux under nitrogen for 15 hours and then cooled in ice bath the

![Fig. 1. Structures of mitochondria-targeted nitroxides.](image-url)
precipitate was separated, washed with toluene and purified using column chromatography (silica gel, eluent chloroform-ethanol 50:1). Further purification can be performed by following procedure: the compound was dissolving in chloroform, the solvent was removed under reduced pressure, the resin-like residue was rapidly dissolved in boiling toluene and filtered, the crystalline precipitate formed was filtered off, washed with toluene and dried. The overall yield from 1b was 1.3 g (51%). Pale yellowish crystals m.p. 236–240°C. Found: C, 68.01; H, 6.80; N, 5.50; Cl 6.96; P 5.70. Calculated for C_{29}H_{35}N_2O_2ClP: C, 68.29; H, 6.92; N, 5.49; Cl 6.95; P 6.07%; ν_{max} (KBr)/cm^{-1} 3424 br, 3158 br, 2974, 2928, 2874, 1669, 1560, 1484, 1460, 1397, 1362, 1324, 1146, 1112, 756, 738, 716.

Similarly, 3-(2-(triphenyphosphonio)acetamido)-2,2,5,5-tetramethylpyrroloidin-1-oxyl (mCP2) was prepared from 2a: the overall yield from 1a was 1.7 g (60%). Pale yellowish crystals m.p. 211–214 °C. Found: C, 68.05; H, 6.71; N, 5.62; Cl 7.65; P 6.19. Calculated for C_{28}H_{33}N_2O_2ClP: C, 67.80; H, 6.71; N, 5.65; Cl 7.15; P 6.24%; ν_{max} (KBr)/cm^{-1} 3425 br, 3182 br, 2974, 2883, 2772, 1670, 1558, 1439, 1365, 1330, 1112, 748, 720, 690, 508, 483.

**Cell culture**

Human aortic endothelial cells (HAEC) were purchased from Lonza (Chicago, IL) and cultured in EGM-2 medium supplemented with 2% FBS but without antibiotics. On the day before the study, the FBS concentration was reduced to 1%. Cells were washed with Krebs-Hepes buffer prior experiments. Cellular accumulation of mitochondria-targeted nitroxides was determined by EPR analysis of cellular pellet following 70 minutes incubation of HAEC with 0.1 μM nitroxide solution in Krebs-Hepes buffer at 37 °C. Cells were collected by centrifugation at 2500 G for 10 minutes at 4 °C and transferred into 50 μl capillary tube for immediate EPR analysis. Concentration of nitroxide in cell pellet was measured with initial nitroxide level in buffer. Cellular reduction of nitroxides into EPR silent hydroxylamine was measured by following time-dependent decay of nitroxide EPR signal in the cellular pellet. In order to test the antioxidant protection of mitochondrial function we pre-incubated HAEC with various concentrations of nitroxides for 15 minutes and then treated with 0.1 mM H_2O_2 or saline as a vehicle. Mitochondrial respiration was measured after additional 60 minutes incubation in cellular pellet placed in 0.25 ml Krebs-Hepes buffer using Clark electrode (Oxygraph, Hanstech).

**Animal experiments**

Hypertension was induced by angiotensin II (0.7 mg/kg/day) as described previously [25] using 2–3 month old male C57Bl/6J mice. Seven days after saline or angiotensin II minipump placement, mice received a second minipump for infusion of saline as vehicle, mCP1 or mCP2 as described in the figure legend. Blood pressure was monitored using either the tail cuff method or telemetry as previously described [26,27]. All procedures were performed according to guidelines and approved by IACUC at Vanderbilt University.

**ESR experiments**

All ESR samples were placed in 50-μl glass capillaries (Corning, New York, NY). ESR spectra were recorded using an EMX ESR spectrometer (Bruker Biospin Corp., Billerica, MA) and a super high Q microwave cavity at room temperature. The ESR settings for field-scan experiments with the spin probe CAT1H were as follows: field sweep, 70 G; microwave frequency, 9.82 GHz; microwave power, 20 mW; modulation amplitude, 0.7 G; conversion time, 41 ms; time constant, 164 ms; and receiver gain, 1 × 10^{5} (n = 4 scans). The rates of H_2O_2 production were determined by monitoring the amplitude of the low field component of the ESR spectrum of CAT1-nitroxide with the following settings: field sweep, 60 G; microwave frequency, 9.46 GHz; microwave power, 20 mW; modulation amplitude, 2 G; conversion time, 1311 ms; time constant, 5243 ms; and receiver gain, 1 × 10^{5}. ESR experiments were repeated at least three times.

**Statistics**

Experiments were analyzed using the Student Neuman Keuls post-hoc test and analysis of variance (ANOVA). P levels < 0.05 were considered significant.

**Results**

**Synthesis and physical properties of mCP1 and mCP2**

In this work we have developed synthesis of two new pyrrolidine mitochondria targeted nitroxides (Fig. 2). The nitroxides mCP1 and mCP2 were synthesized from 3-amino- and 3-amino-methyl-proxyls via chloroacetylation and treatment with triphenylphosphine according to the general Scheme 1 in analogy to the procedure previously described for mitoTEMPO (mt) [24].

**Reaction of O_2•− with mCP1 and mCP2**

We have investigated reaction of O_2•− with mitochondria targeted nitroxides using xanthine oxidase O_2•− generating system and cytochrome c as was previously described by Murphy et al. [28] Cytochrome c is rapidly reduced by O_2•− into ferrocytochrome c (8 × 10^{-5} M^{-1}s) [29] and this reaction is inhibited in the presence of nitroxide radicals. Indeed, O_2•− mediated cytochrome c reduction was inhibited in dose-dependent manner in the presence of mCP1, mCP2 and mitoTEMPO (Fig. 3A). The rate constants of O_2•− reaction with nitroxides were estimated from the linear regression of inhibition of cytochrome c reduction (Fig. 3B) expressed as (V_0/V-1), where V and V_0 are the rates of cytochrome c reduction in the presence or absence of nitroxide. The slope of this linear regression is proportional to the rate constants of O_2•− reaction with nitroxides. The sharpest slope for mitoTEMPO indicates the highest rate of reaction with O_2•− compared with mCP1 and mCP2. Taking into account the concentration of cytochrome c (50 μM) we have estimated the nitroxide rate constants as...
2.5 \times 10^5 \text{M}^{-1}\text{s}^{-1} (\text{mitoTEMPO}), 7.6 \times 10^4 \text{M}^{-1}\text{s}^{-1} (\text{mCP1}) and 3.2 \times 10^4 \text{M}^{-1}\text{s}^{-1} (\text{mCP2}). These data support fast reaction between new mitochondria targeted nitroxides and \( \text{O}_2^{-}\), however \text{mCP1} and \text{mCP2} react with \( \text{O}_2^{-}\) by 3- and 7-fold slower compared with mitoTEMPO.

Cellular accumulation and protection of mitochondrial respiration by \text{mCP1} and \text{mCP2}

We have previously shown rapid accumulation of mitoTEMPO in intact endothelial cells [6]. In this work we have compared cellular accumulation of \text{mCP1}, \text{mCP2}, mitoTEMPO and its untargeted analog TEMPOL. EPR analysis of intact HAEC incubated with 5 \text{µM} nitroxides showed significant increase in nitroxide accumulation in cellular pellet. It was found that cellular level of \text{mCP1} and \text{mCP2} was increased by 37-fold compared with extracellular nitroxide concentration (Fig. 4A). Cellular accumulation of mitoTEMPO was 25-fold while concentration of untargeted TEMPO was increased only by 4.6-fold vs buffer. These data demonstrate improved cellular accumulation of \text{mCP1} and \text{mCP2} compared with mitoTEMPO.

To estimate the protective activity of the new mitochondria targeted proxyl nitroxides we studied their effect on HAEC under \( \text{H}_2\text{O}_2\)-induced mitochondrial oxidative stress recently described by Kalyanaraman group [19]. We have previously shown that \( \text{H}_2\text{O}_2\) stimulates production of mitochondrial \( \text{O}_2^{-}\) [7] and therefore \( \text{O}_2^{-}\) scavengers should protect mitochondria. Indeed, it was found that treatment of intact HEAC with low dose of \( \text{H}_2\text{O}_2\) reduced mitochondrial respiration by 2.4-fold while supplementation with \text{mCP1}, \text{mCP2} and mitoTEMPO protected respiration in a dose-dependent manner (Fig. 4B). Mito-TEMPO was more efficient at low concentration (30 nM) compared with \text{mCP1} and \text{mCP2} which likely due to higher reactivity of mitoTEMPO with \( \text{O}_2^{-}\). However, higher doses of nitroxides (100 nM) showed similar protection of cellular respiration.
Cellular reduction of mitochondria targeted nitroxides

Nitroxides can be susceptible to bioreduction into EPR silent hydroxylamine form [20]. The increased resistance to bioreduction may contribute to higher cellular level of mCP1 and mCP2 compared with mitoTEMPO (Fig. 5A). We therefore investigated bioreduction of mitochondria targeted nitroxides. It was found that incubation of intact endothelial cells with mitoTEMPO for 40 minutes reduced intensity of EPR spectra by 41%. Interestingly, incubation mCP1 and mCP2 reduced EPR signals by 13% and 18%, correspondingly (Fig. 5A). Analysis of EPR spectra showed that the reduction rates of mCP1 and mCP2 were 2–3-fold slower compared with mitoTEMPO (Fig. 4B). These data are in line with higher resistance to reduction of proxyl nitroxides compared to TEMPO derivatives.

In vivo treatment with mCP1 and mCP2 after onset of hypertension

Increased vascular $O_2^{-}$ production has been implicated in the pathogenesis of endothelial dysfunction and hypertension [30,31]. We have previously shown that mitoTEMPO supplementation attenuates development of hypertension [6]. Furthermore, treatment of animals after onset of hypertension reduced vascular oxidative stress, improved NO-mediated vasodilatation and reduced blood pressure [6]. Meanwhile, we do not know if other mitochondria targeted nitroxides will reduce blood pressure or antihypertensive effect is specific to mitoTEMPO. We therefore performed additional studies in which mCP1 and mCP2 were administered after the onset of angiotensin II-induced hypertension. Following 9 days of angiotensin II infusion (0.7 mg/kg/day) systolic blood pressure reached 150 mm Hg (Fig. 6A). The subsequent addition of mCP1 or mCP2 (1.4 mg/kg/day) using osmotic mini-pump resulted in a time-dependent decrease of blood pressure. Infusion of mCP1 and mCP2 significantly reduced blood pressure to 133 ± 5 mm Hg and 129 ± 6 mm Hg while blood pressure in mice infused with saline (vehicle) have continued to rise and reached 163 ± 5 mm Hg at day 14 (Fig. 6A). Of note, treatment of healthy control mice with mitochondria targeted nitroxides did not affect basal blood pressure (101 mm Hg) because $O_2^{-}$ does play a role in the regulation of blood pressure in normotensive subjects [6]. These data indicate that mCP1 and mCP2 provide antihypertensive effect similar to mitoTEMPO which we have previously described [6].

In order to compare time-dependent antihypertensive effects of mitochondria-targeted nitroxides we have treated hypertensive mice with daily i.p. injection of saline (vehicle), or 1.4 mg/kg mCP1, mCP2 or mitoTEMPO and measured blood pressure before and after injection. It was found that i.p. treatment with nitroxides acutely reduced blood pressure however this antihypertensive effect was slightly reduced next day after injection (Fig. 6B). Meanwhile the daily treatments increased antihypertensive effect in a time-dependent fashion. Interestingly, i.p. treatment with mCP1 and mCP2 provide antihypertensive effect similar to mitoTEMPO (Fig. 6B).

The above studies showing that mCP1 and mCP2 can reduce hypertension do not provide insight into whether antihypertensive effect was associated with reduced $O_2^{-}$ level and improved vascular function. Therefore, additional studies were performed of the antioxidant effects of mCP1 and mCP2. Vascular $O_2^{-}$ production in isolated aortic vessels from hypertensive

![Fig. 5.](image-url) EPR spectra of intact endothelial cells incubated with mCP1, mCP2 and mitoTEMPO (A). Rates of cellular nitroxide reduction during incubation at 37 °C (B). Results represent mean ± SEM for 4–6 repeats per group. *P < 0.01 vs mitoTEMPO, **P < 0.05 vs mitoTEMPO.
mice after onset of angiotensin II induced hypertension was measured using fluorescent probe DHE and HPLC analysis of $O_2^{-}\cdot$ specific product 2-hydroxyethidium (Fig. 7A) [32]. Both mCP1 and mCP2 reduced vascular $O_2^{-}\cdot$ and blood pressure similarly to previously report for mitoTEMPO [6] which support pharmacological activity in treatment of vascular oxidative stress by proxyl-based mitochondria-targeted antioxidants.

Increased $O_2^{-}\cdot$ production in vessels is leading to inactivation of endothelial nitric oxide and vasoconstriction which contributes to hypertension [30]. We hypothesized that mitochondria-targeted nitroxides will improve vascular nitric oxide level since these treatments reduced vascular $O_2^{-}\cdot$. In order to test this hypothesis we measured vascular nitric oxide in aortic vessels using electron spin resonance and specific nitric oxide spin trap Fe(DETC)$_2$ as we have previously described [33]. It was found that treatment of hypertensive mice treated with mCP1 or mCP2 prevented oxidation of nitric oxide and significantly improved vascular nitric oxide level (Fig. 7B).

### Discussion

In this work we have synthesized novel proxyl-based mitochondria-targeted nitroxides mCP1 and mCP2 and studied their antioxidant properties. The cellular penetration of these nitroxides is driven by the lipophilic triphenyl cation and therefore it is likely to be similar for proxyl and TEMPO based mitochondria targeted nitroxides. Our data however show significant difference in the intracellular reduction of mCP1, mCP2 and mitoTEMPO (Fig. 5). These nitroxides were present in intact cells at higher concentration compared with mitoTEMPO (Fig. 4A) which is in line with greater bioreduction resistance of proxyl based nitroxides (Fig. 5). The advantageous cellular accumulation of mCP1 and mCP2 however was counteracted by reduced reactivity with $O_2^{-}\cdot$ compared with mitoTEMPO (Fig. 3) which can eliminate the potential advantages of proxyl-based nitroxides. Indeed, both proxyl- and TEMPO-based mitochondria targeted nitroxides showed similar protection of respiration in $H_2O_2$-treated endothelial cells (Fig. 4).

Furthermore, our in vivo study in angiotensin II-infused mice...
showed that antihypertensive and antioxidant effects of mCP1 and mCP2 were not significantly different from mito-TEMPO (Figs. 6 and 7). Our data also show that mito-TEMPO is not the only mitochondria-targeted nitroxide exhibiting antihypertensive effect and studies of analogs such as mCP1 and mCP2 may help in optimization of chemical structure of mitochondria-targeted nitroxides for improved, lipophilia, antioxidant efficacy and pharmacokinetics.

Antihypertensive effects of TEMPOL and other untargeted nitroxides has been previously shown [34]. Acute treatment of hypertensive animals with TEMPOL caused dose-dependent reductions in blood pressure which was accompanied with vasodilation, increased nitric oxide, reduced sympathetic activity and enhanced potassium channel conductance in blood vessels and neurons [34]. We have proposed that mitochondria-targeted SOD mimetic will specifically reduce mitochondrial O$_2^•$ and reduce vascular oxidative stress and improve endothelium dependent relaxation. Indeed, we have previously demonstrated that mitoTEMPO attenuated hypertension in dose-dependent manner, reduced production of mitochondrial O$_2^•$ and H$_2$O$_2$, increased endothelial NO and improved vasodilatation while similar dose of TEMPO was not effective [6]. Importantly, we found that targeting of SOD mimetic to mitochondria provided beneficial effects at a dose 1000-fold lower than previously reported for TEMPOL [6]. In this work we show similar activity of other mitochondria-targeted mCP1 and mCP2 therefore representing new antihypertensive agents that could add to the currently available therapeutic armamentarium.

These studies confirmed an important role of mitochondrial O$_2^•$ in the hypertension and demonstrated that mitochondria-targeted nitroxides could have therapeutic benefit in treatment of hypertension.

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These studies confirmed an important role of mitochondrial O$_2^•$ in the hypertension and demonstrated that mitochondria-targeted nitroxides could have therapeutic benefit in treatment of hypertension.
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