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Selective Phosphodiesterase 1 Inhibition Ameliorates Vascular Function, Reduces Inflammatory Response, and Lowers Blood Pressure in Aging Animals

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ABSTRACT

Diminished nitric oxide-cGMP–mediated relaxation plays a crucial role in cardiovascular aging, leading to decreased vasodilation, vascular hypertrophy and stiffening, and ultimately, cardiovascular dysfunction. Aging is the time-related worsening of physiologic function due to complex cellular and molecular interactions, and it is at least partly driven by DNA damage. Genetic deletion of the DNA repair enzyme ERCC1 endonuclease in *Ercc1*$^{\Delta/-}$ mice provides us an efficient tool to accelerate vascular aging, explore mechanisms, and test potential treatments. Previously, we identified the cGMP-degrading enzyme phosphodiesterase 1 as a potential treatment target in vascular aging. In the present study, we studied the effect of acute and chronic treatment with ITI-214, a selective phosphodiesterase 1 inhibitor on vascular aging features in *Ercc1*$^{\Delta/-}$ mice. Compared with wild-type mice, *Ercc1*$^{\Delta/-}$ mice at the age of 14 weeks showed decreased reactive hyperemia, diminished endothelium-dependent and -independent responses of arteries in organ baths, carotid wall hypertrophy, and elevated circulating levels of inflammatory cytokines. Acute ITI-214 treatment in organ baths restored the arterial endothelium-independent vasodilation in *Ercc1*$^{\Delta/-}$ mice. An 8-week treatment with 100 mg/kg per day ITI-214 improved endothelium-independent relaxation in both aorta and coronary arteries, at least partly restored the diminished reactive hyperemia, lowered the systolic and diastolic blood pressure, normalized the carotid hypertrophy, and ameliorated inflammatory responses exclusively in *Ercc1*$^{\Delta/-}$ mice. These findings suggest phosphodiesterase 1 inhibition would provide a powerful tool for nitric oxide-cGMP augmentation and have significant therapeutic potential to battle arteriopathy related to aging.

SIGNIFICANCE STATEMENT

The findings implicate the key role of phosphodiesterase 1 in vascular function and might be of clinical importance for the prevention of mortalities and morbidities related to vascular complications during aging, as well as for patients with progeria that show a high risk of cardiovascular disease.

Introduction

Aging is the time-related worsening of physiologic function due to complex cellular and molecular interactions. The substantial increase in the average human life span brought by improved nutritional, sanitary, and healthcare conditions over the last decades is accompanied by an elevation in the significant age-related cardiac, arterial, and microvascular disease burden in a growing number of countries worldwide. Focusing on the vasculature, such pathologic conditions include worsened vasodilation, increased arterial stiffness, reduced blood flow in small resistance arteries, and dysfunctional endothelium (Donato et al., 2018; Fajemiroye et al., 2018; Oue et al., 2018).

The onset of cardiovascular disease (CVD) is triggered by vascular alterations characterized by impaired vasodilation and the overproduction of inflammatory markers. A strong body of evidence from epidemiologic studies shows that aging itself is the major independent risk factor for cardiovascular diseases (Versari et al., 2009; Widmer and Lerman, 2014; Bautista Niño et al., 2015; de Almeida et al., 2017). Unlike risk

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ABBREVIATIONS: ACh, acetylcholine; CRC, concentration-response curve; CVD, cardiovascular disease; EDH, endothelium-dependent hyperpolarization; ERCC, excision repair cross-complementing; IFN-γ, interferon-γ; IL, interleukin; L-NAME, L-NG-nitro arginine methyl ester; MSD, Meso Scale Discovery; NO, nitric oxide; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; PDE, phosphodiesterase; SNP, sodium nitroprusside; TNF-α, tumor necrosis factor-α; VSMC, vascular smooth muscle cell; WT, wild type.

factors such as dyslipidemia and hypertension, for which suitable models exist to study their primary risk contributions to vascular dysfunction and response to prevention or intervention maneuvers, aging lacks such models and has thus been harder to study. This limitation was recently overcome by the discovery that accumulating DNA damage is a central causative mechanism for aging, along with the development of accelerated-aging mouse models based on genetically reduced DNA repair (Wu et al., 2017; Golshiri et al., 2020a). In several of these models, risk factor–free accelerated vascular aging was demonstrated. One such model, the *Ercc1*$^{\Delta/-}$ mouse, lacks the proper function of ERCC1 (an endonuclease that plays a role in the repair of helix-distorting DNA adducts, crosslinks, and homologous recombination) and develops all major nonatherosclerotic vascular aging features found in elderly humans (Wood, 2010; Faridounnia et al., 2018). This includes increased blood pressure and vascular stiffness and reduced vasodilation (Durik et al., 2012). The model has been used as a convenient tool to test the effects of dietary and pharmacological interventions. We have used this model to explore mechanisms of aberrant nitric oxide (NO)-cGMP signaling in smooth muscle cells, which plays a central role in vascular aging (Wu et al., 2017; Golshiri et al., 2020a).

In aging-related CVD, the second messenger signaling molecules 3′,5′-cyclic adenosine and guanosine monophosphate (cAMP, cGMP) play a vital role, being involved in dysfunctional vasomotor activity, vessel tone, permeability, proliferation, and fibrosis. Both cAMP and cGMP are also implicated as negative mediators of the transformation of quiescent cardiac fibroblasts to active myofibroblasts (Naka et al., 1996; Fukuhara et al., 2005; Insel et al., 2012; Ataei Ataabadi et al., 2020; Golshiri et al., 2020b). The levels of these second messengers are determined by the balance between their formation and the phosphodiesterase (PDE) enzymes that catalyze their hydrolysis and inactivation. PDE's consist of 11 main members expressed as more than 100 isoforms that are encoded by at least 21 distinct genes and are classified based on substrate specificities and regulatory factors. The cell- and tissue-specific expression of PDE's provide a great opportunity for better organ targeting and more specific therapeutic interventions (Lugnier, 2006; Ataei Ataabadi et al., 2020). In *Ercc1*$^{\Delta/-}$ mice, in combination with human studies, we identified PDE1 as a potential contributor to vascular aging (Bautista Niño et al., 2015). The PDE1 family members are dual-substrate enzymes that hydrolyze and inactivate both cGMP and cAMP and are activated by a Ca^{2+}/calmodulin-binding domain (Ataei Ataabadi et al., 2020). PDE1 is expressed as three identified isoforms (1A, 1B, and 1C). It is highly expressed in the smooth muscle cell and appears to contribute to the lack of vasodilation capacity in *Ercc1*$^{\Delta/-}$ arteries, as demonstrated with the nonselective PDE inhibitor vinpocetine (Bautista Niño et al., 2015). The potentially important role of PDE1 in vascular function and its correlation with several risk factors in cardiovascular diseases, including heart pressure overload, hypertrophy, pulmonary arterial hypertension, and postmyocardial infarction, have been highlighted in several key studies (Murray et al., 2007; Miller et al., 2009, 2011; Hashimoto et al., 2018; Dey et al., 2020). Systematic research employing potent and selective PDE1 inhibitors in animal models of the accelerated phenotype of aging is currently lacking. However, the recently developed highly selective PDE1 inhibitor ITI-214, which possesses a similar affinity for all three isoforms of PDE1 and is well tolerated in human safety studies, is under clinical development for the treatment of heart failure and neurodegenerative disease and has opened new opportunities (Li et al., 2016; Snyder et al., 2016; Wennogle et al., 2017; Hashimoto et al., 2018; Pekcec et al., 2018).

Considering our recent findings on the role of PDE1 in reduced vasodilation in *Ercc1*$^{\Delta/-}$ accelerated-aging mice and findings of others in healthy mice (Bautista Niño et al., 2015), we hypothesized that selective PDE1 inhibition could reduce blood pressure, improve vascular function, and ameliorate the resistance in small arteries through elevation of cAMP and cGMP second messengers. In this study, we demonstrate that acute and chronic treatment with the PDE1 selective inhibitor ITI-214 can improve age-dependent vascular function and reduces the elevated level of inflammatory cytokines in plasma, suggesting a potential general mechanism of improved signaling through inhibition of PDE1.

Material and Methods

Drugs and Reagents. All chemicals and reagents were purchased from Sigma-Aldrich, and ITI-214 was provided by Intra-Cellular Therapies.

Animals. *Ercc1*$^{\Delta/-}$ and *Ercc1*$^{+/+}$ F1 mice with a hybrid C57BL6J::FVB background were generated by crossbreeding of parents with a pure C57BL6J and FVB background. The genotype and phenotype features of the model have been described before (Weeda et al., 1997; Vermeij et al., 2016). In short, in *Ercc1*$^{\Delta/-}$ mice, the ERCC1 gene is not completely deleted but is partially inactivated by introducing a carboxy-terminal UvrC-homologous region into the encoded protein. The encoded protein contains a seven-amino-acid carboxy-terminal truncation (equivalent to deleting six residues of the human protein). The ERCC1 knockout mice all die before day 38, whereas the maximum life span of *Ercc1*$^{\Delta/-}$ mice is considerably longer (6 months). The hybrid background of the experiment mice prevents strain-specific phenotypes. Breeding was performed at the Erasmus MC animal facility. Mice were housed in individually ventilated cages in a controlled environment (20–22°C, 12-hour light/dark cycle) with access to normal chow and water ad libitum. The animals were weighed and visually inspected every day to warrant their well-being. All animal studies were performed in accordance with the Principles of Laboratory Animal Care and with the guidelines approved by the independent Dutch Animal Ethical Committee.

Study Design. In total, 34 *Ercc1*$^{\Delta/-}$ mice at the age of 6 weeks and 28 of their WT littermates (*Ercc1*$^{+/+}$) of the same age were randomized into four groups: one group of *Ercc1*$^{\Delta/-}$ mice and one of the WT were given normal drinking water; another group of *Ercc1*$^{\Delta/-}$ and WT mice received the PDE1 inhibitor ITI-214 (100 mg/kg per day) via drinking water for 8 weeks. Both male and female animals were used. Blood pressure and superficial blood flow were measured 1 week prior to sacrifice. At the age of 14 weeks, the mice were sacrificed, the tissues were collected and snap-frozen, and wire myography and pressure myography experiments were done.

Sacrifice and Tissue Harvest. At the age of 14 weeks, the animals were sacrificed by cardiac puncture and lethal blood withdrawal from the vena cava under anesthesia. Blood was centrifuged at 2500 rpm at 4°C for 10 minutes, and plasma was collected and stored at −80°C. The thoracic aorta and coronary and carotid arteries were carefully isolated and stored in cold Krebs-Henseleit buffer solution for organ bath experiments.

Wire Myography. The thoracic aorta was isolated and cleaned in cold oxygenated (with 95% O_2 and 5% CO_2) Krebs-Henseleit buffer solution (in mmol/L: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 8.3; pH 7.4) for ex vivo wire myography experiments. The 2-mm segments of the thoracic aorta were mounted in 6-ml chambers of wire myography device (Danish

Myograph Technology, Aarhus, Denmark). After the normalizing procedure, the maximum contractile responses were determined using 100 mmol/L KCl. After four-times washing steps with a 5-minute interval for each step, 30 nmol/L U46619 or 30 mmol/L KCl was applied to preconstrict the vessel segments and evaluate the relaxation concentration-response curves (CRCs) to acetylcholine (ACh) and sodium nitroprusside (SNP) (respectively). The 2-mm coronary segments were also mounted following the same procedure, the maximum contractile responses were determined using 60 mmol/L KCl, and CRCs to ACh and SNP were performed after preconstriction with 30 nmol/L U46619. L-NAME 100 μmol/L, TRAM34 10 μmol/L, and apamin 100 nmol/L were given 10 minutes before U46619 to investigate the involvement of NO and endothelium-dependent hyperpolarization (EDH) pathway in the relaxation responses (Golshiri et al., 2020a). To assess the acute effects of PDE1 inhibition, 100 nmol/L ITI-214 was added to the organ bath 15 minutes before preconstriction.

Analysis of ITI-214 Levels in Plasma. ITI-214 levels were determined in the mouse plasma using liquid-liquid extraction with subsequent high-performance liquid chromatography and tandem mass spectrometry detection. A nominal concentration range of 1–1000 ng/ml for ITI-214 was chosen to quantitate the study samples. Samples were kept frozen at −70 ± 10°C prior to analysis. A 20-μl matrix aliquot was diluted with 100 μl water, followed by extraction using acetonitrile fortified with 1 ng/ml of internal standard and 1% of formic acid. Analytes were isolated through an Ostro sample preparation plate. The eluate was injected into a Shimadzu HPLC system and detected via MS/MS using positive ion electrospray through a Sciex API 6500 mass spectrometer. A linear, 1/concentration weighted, least-squares regression algorithm was used to quantitate unknown samples.

cGMP Measurement in Plasma. The plasma level of cGMP was measured using an ELISA kit from Enzo Life Sciences (Farmingdale, NY) and versa max microplate reader. In total, 20 μl of the plasma was used to run the acetylated version of the kit according to the manufacturer protocol. All standards and samples were run in duplicate.

Blood Pressure and Vasodilator Function (In Vivo). A laser Doppler perfusion imaging system (Perimed, PeriScan PIM 3 System) was used to assess in vivo hind leg vasodilator function, as described before. In short, mice were anesthetized by 2.8% isoflurane/O_2 ventilation (Penlon, Sigma Delta vaporizer) while keeping the body temperature at 37.0°C. The hind leg, while kept in a fixed position, was occluded for 2 minutes with a tourniquet. Upon release of the tourniquet, reactive hyperemia was measured for 10 minutes. Results were expressed as the area under the response curve (Durik et al., 2012; Bautista Niño et al., 2015).

The in vivo blood pressure was measured in conscious animals by an experienced technician using the tail-cuff technique (CODA High-Throughput device from Kent Scientific) after five daily sessions: four training sessions and a subsequent measurement session to record 40 measurement cycles. The average of the valid cycles was used for comparison (Golshiri et al., 2020a).

Pressure Myography. After the sacrifice, the carotid arteries were isolated from 14-week-old *Ercc1*$^{\Delta/-}$ and WT animals and cleaned from all the fat tissues. The carotid arteries were mounted in pressure myograph (Danish Myograph Technology, Aarhus, Denmark) in calcium-free buffer (in mmol/L: NaCl 120, KCl 5.9, EGTA 2, $MgCl_2$ 3.6, NaH_2PO_4 1.2, glucose 11.4, $NaHCO_3$ 26.3; pH 7.4) to assess the passive properties of the vessels. The intraluminal pressure of the vessel was increased stepwise by 3-minute, 10-mm Hg steps, starting from 10 mm Hg and ending at 120 mm Hg. At the end of each step, the lumen diameter and wall thickness of the vessel were measured (Durik et al., 2012).

Analysis of Plasma Cytokine Levels. Plasma protein levels of IL-1β, IL-2, IL-6, IL-10, TNF-α, and IFN-γ were measured using a V-Plex Meso Scale Discovery (MSD) Multiplex spot assay Mouse Neuroinflammation 1 panel. All samples were diluted at a ratio of 1:4 with diluent 41 (provided in the MSD kit). Samples and standards were run in duplicate or triplicate according to manufacturer instructions and analyzed with MSD Discovery Workbench software (Meso Scale Discovery, Gaithersburg, MD).

Data Analysis. Relaxation to ACh and SNP are expressed relative to the contraction produced by 30 nmol/L U46619 or 30 mmol/L KCl, which were set at 100% in each individual aortic or coronary rings. Data are shown as the percentage of relaxation, expressed as the mean ± S.E.M. The number of each individual experiment is shown for each of the rings. Statistical analysis was conducted using IBM SPSS statistics (IBM Corporation, version 25) and (GraphPad Prism, version 8.0.1; GraphPad Software Inc., San Diego, CA). Data were analyzed using Student's paired t test and general linear model repeated measurements. P values less than 0.05 were considered significant.

Results

ITI-214 Acutely Improves the Diminished Aortic NO Responsiveness in *Ercc1*$^{\Delta/-}$ Mice. In agreement with previous studies in this model (Bautista Niño et al., 2015), SNP responsiveness is diminished in *Ercc1*$^{\Delta/-}$ mice versus WT. The acute addition of ITI-214 to the organ bath enhanced the SNP response in *Ercc1*$^{\Delta/-}$ mice (Fig. 1).

IT-214 Levels in the Blood Are in the Normal Range during Chronic Treatment. To evaluate the amount of drug consumption by drinking water and confirming sufficient drug intake and drug concentration in the blood, the plasma level of ITI-214 was measured after 8 weeks of treatment (100 mg/kg per day). The mean plasma concentration of ITI-214 is about 140 ng/ml, which is comparable to the 60-ng/ml concentration we have used in our acute study in the organ baths. It also corresponds to free plasma concentrations of 1 to 2 nmol/L ITI-214 (due to >99% plasma protein bound), which is above the K_i for recombinant PDE1 but far below the K_i of other PDEs (Hashimoto et al., 2018).

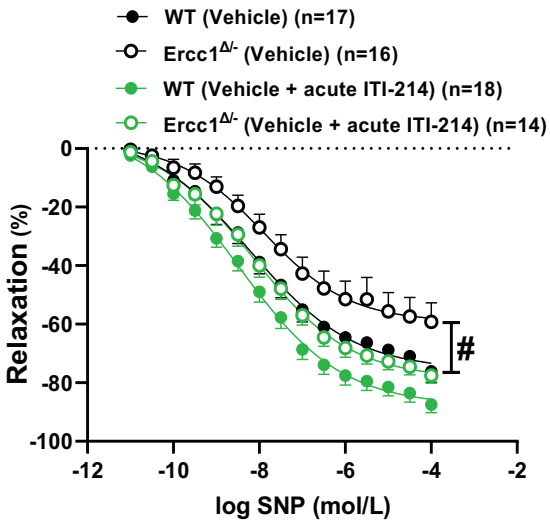

Fig. 1. Endothelium-independent responses in isolated aortic rings from *Ercc1*$^{\Delta/-}$ mice (open circles) versus rings from WT littermate (filled circles) measured ex vivo in small wire organ baths; the acute effect of ITI-214 administration to organ bath in WT and *Ercc1*$^{\Delta/-}$ mice is in green lines. Relaxations are calculated relative to the contraction produced by KCl 30 mmol/L in each ring, which are set at 100%. Values are expressed as means ± S.E.M.; $^{\#}P < 0.05$, two-way ANOVA followed by Bonferroni's post hoc test on E_{max}; *Ercc1*$^{\Delta/-}$ (vehicle) versus WT (vehicle) and *Ercc1*$^{\Delta/-}$ (vehicle) versus *Ercc1*$^{\Delta/-}$ (vehicle + acute ITI-214).

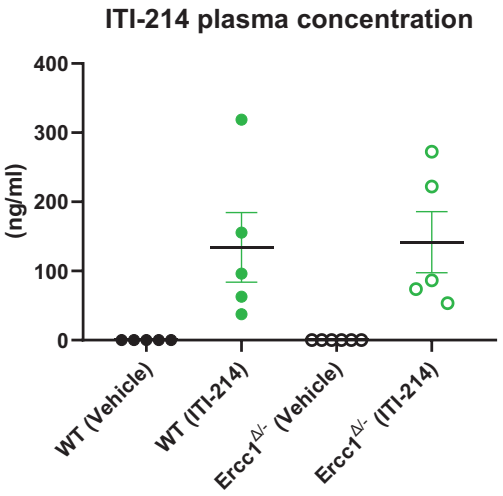

Fig. 2. The plasma concentration of the ITI-214 in both WT and *Ercc1*$^{\Delta/-}$ mice after 8 weeks of treatment.

ITI-214 Treatment Restores the Endothelium-Independent but Not the Endothelium-Dependent Response of the Aorta in *Ercc1*$^{\Delta/-}$ Mice. The well known diminished aortic ACh response in *Ercc1*$^{\Delta/-}$ mice versus WT was not affected by ITI-214 treatment (Fig. 3A), yet it did restore the disturbed response of the aorta to the endothelium-independent vasodilator SNP in *Ercc1*$^{\Delta/-}$ mice (Fig. 3B).

ITI-214 Does Not Alter the Contribution of NO or EDH during Exposure of the Aorta to Acetylcholine. To explore the signaling pathways underlying the decreased endothelium-dependent vasodilation, we studied the contribution of NO and EDH to the ACh relaxation in aortic rings using the NO inhibitor L-NAME and the EDH inhibitor cocktail TRAM34 and apamin. In both vehicle-treated *Ercc1*$^{\Delta/-}$ and WT mice, L-NAME significantly decreased the ACh response (Fig. 4, A and B). The effect of L-NAME was less pronounced in *Ercc1*$^{\Delta/-}$ than in WT. TRAM34/apamin had no effect in *Ercc1*$^{\Delta/-}$ mice and nonsignificantly reduced the ACh response in WT mice when added on top of L-NAME. The residual response in the presence of both L-NAME + TRAM34/apamin was not different between *Ercc1*$^{\Delta/-}$ and WT. Altogether, we demonstrate that the NO-mediated relaxations were hampered in *Ercc1*$^{\Delta/-}$ versus WT. ITI-214 did not change the relative contribution of endothelium-derived NO and EDH in both WT or *Ercc1*$^{\Delta/-}$ (Fig. 4, C and D).

ITI-214 Treatment Restores the Endothelium-Independent but Not the Endothelium-Dependent Response of the Coronary Artery in *Ercc1*$^{\Delta/-}$ Mice. To explore the role of PDE1 in the disturbed vasodilatory responses of small arteries, we also studied coronary arteries of vehicle- and ITI-214–treated mice. The difference in vasodilator response in *Ercc1*$^{\Delta/-}$ mice versus WT littermates (Fig. 5, A and B) of coronary arteries was far more pronounced compared with aorta segments (Figs. 1A and 2A). Similar to the aorta, chronic ITI-214 treatment did not improve the ACh response (Fig. 5, C and D), whereas it fully restored the response to SNP in *Ercc1*$^{\Delta/-}$ mice (Fig. 5, E and F).

ITI-214 Treatment Does Not Change the cGMP Level in Plasma. The chronic treatment with ITI-214 did not change the plasma level of cGMP in both *Ercc1*$^{\Delta/-}$ mice and WT littermates. The SNP responses in *Ercc1*$^{\Delta/-}$ mice and WT littermates can be fully blocked by the addition of the sGC inhibitor ODQ (Fig. 6, A and B).

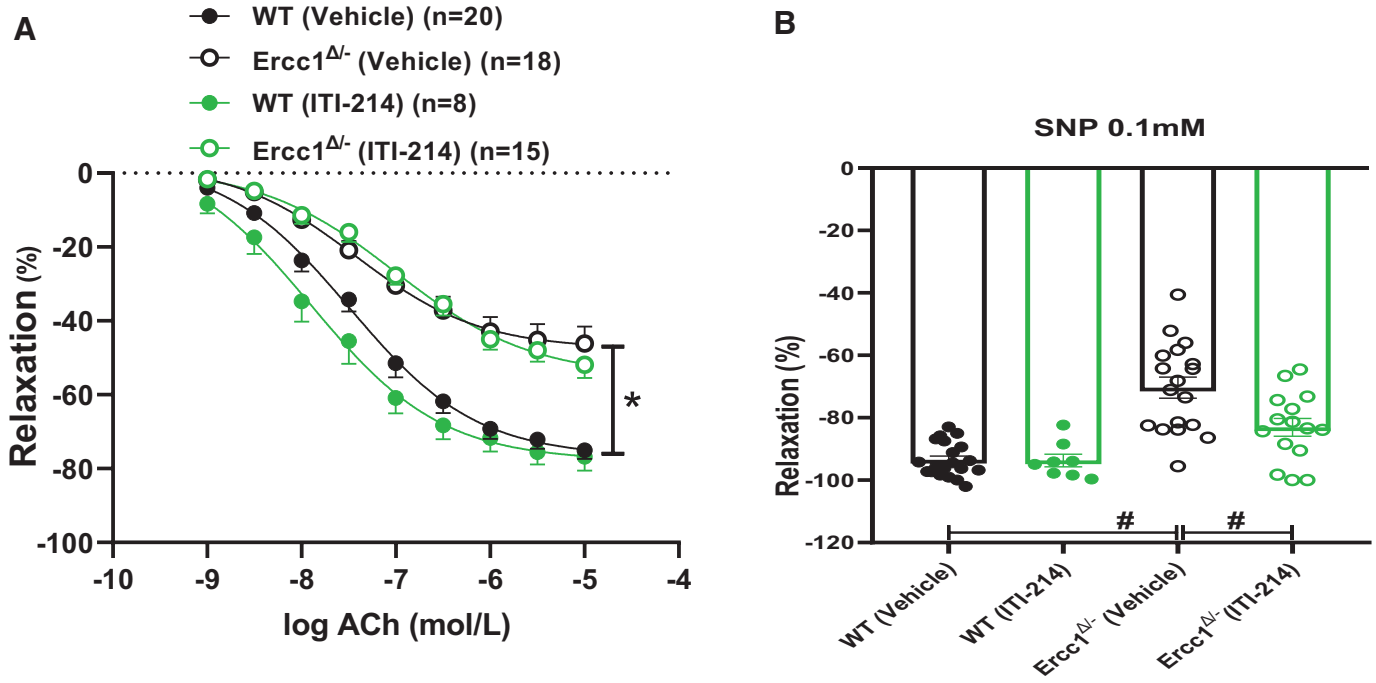

Fig. 3. Endothelium-dependent and -independent responses in isolated aortic rings from *Ercc1*$^{\Delta/-}$ mice (open circles, bars) versus rings from WT littermate (filled circles, bars) (A and B). The endothelium-dependent and -independent effects of 8 weeks of treatment with ITI-214 in drinking water on WT and *Ercc1*$^{\Delta/-}$ mice in green lines/bars, measured in aortic rings in ex vivo organ bath experiments. Data are calculated relative to the precontraction. Values are expressed as means ± S.E.M.; $^{*}P < 0.05$, GLM for repeated measures; $^{\#}P < 0.05$, two-way ANOVA followed by Bonferroni's post hoc test.

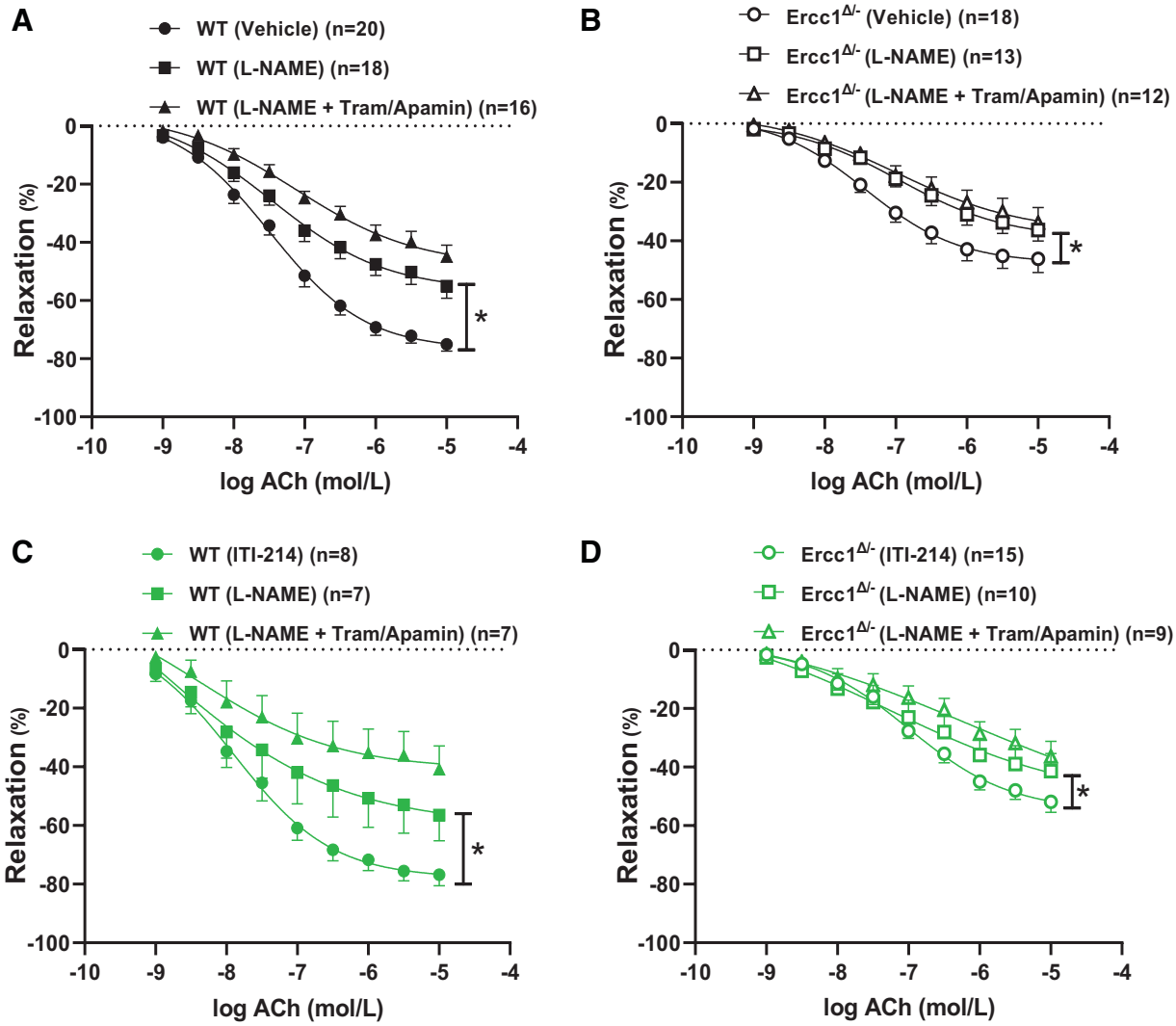

Fig. 4. The impact of the NO synthesis inhibitor L-NAME (100 μmol/L) and EDH inhibitors TRAM34 (10 μmol/L) and apamin (100 nmol/L) on the relaxations induced by ACh in aortic rings precontracted by U46619. A, B: vehicle-treated mice. C, D: ITI-214-treated mice. Relaxations are calculated relative to the contraction produced by U46619 in each ring, which is set at 100%. Values are expressed as means ± S.E.M.; $^*P < 0.05$, GLM for repeated measures.

ITI-214 Treatment Lowers Blood Pressure in *Ercc1*$^{\Delta/-}$ Mice. Blood pressure measured by tail-cuff in conscious mice was identical in untreated *Ercc1*$^{\Delta/-}$ mice (SBP: 117.85 ± 19.98; DBP: 83.35 ± 18.40) and their WT littermates (SBP: 107.21 ± 15.37; DBP: 73.48 ± 17.83). Treatment with ITI-214 reduced both systolic and diastolic blood pressure in *Ercc1*$^{\Delta/-}$ mice (SBP: 93.38 ± 10.21; DBP: 64.46 ± 7.99) but not WT (SBP: 108.97 ± 18.19; DBP: 73.30 ± 21.17) (Fig. 7, A and B).

ITI-214 Treatment Normalizes the Increased Wall/Lumen Ratio in Carotid Arteries of *Ercc1*$^{\Delta/-}$ Mice. *Ercc1*$^{\Delta/-}$ showed an increased wall-to-lumen ratio at 100 mm Hg compared with WT (Fig. 7C), which was restored by ITI-214.

ITI-214 Treatment Improves Microvascular Blood Flow in *Ercc1*$^{\Delta/-}$ Mice. Since ITI-214 improved NO-mediated dilation in the coronary artery, a midsized artery, we explored the effect of chronic ITI-214 on microvascular blood flow. Reactive hyperemia to a 2-minute hind leg occlusion, measured by laser Doppler, was significantly lower in *Ercc1*$^{\Delta/-}$ compared with WT (Fig. 7D), whereas it has been partly restored and the difference was no longer present after ITI-214 chronic treatment.

ITI-214 Treatment Lowers the Elevated Levels of Inflammatory Cytokines in *Ercc1*$^{\Delta/-}$ Mice. The plasma levels of IL-1β, IL-6, TNF-α, IL-2, and IL-10 were elevated in *Ercc1*$^{\Delta/-}$ mice versus WT, whereas IFN-γ was reduced. ITI-214 significantly reduced the levels of the former three (Fig. 8).

Discussion

Age-related CVD is the leading worldwide cause of mortality and morbidity among all acquired diseases. Functional and structural alterations in vascular tissue are at the early basis of the etiology of CVD, and characterization and intervention in the pathogenic mechanisms that govern these alterations are key in the development of pharmacotherapy (Ghebre et al., 2016; Donato et al., 2018; Fajemiroye et al., 2018). PDE1 was previously identified by us as a potential drug target in vascular aging to rescue decreased NO-cGMP signaling, a pivotal pathogenic mechanism in the aging cardiovascular

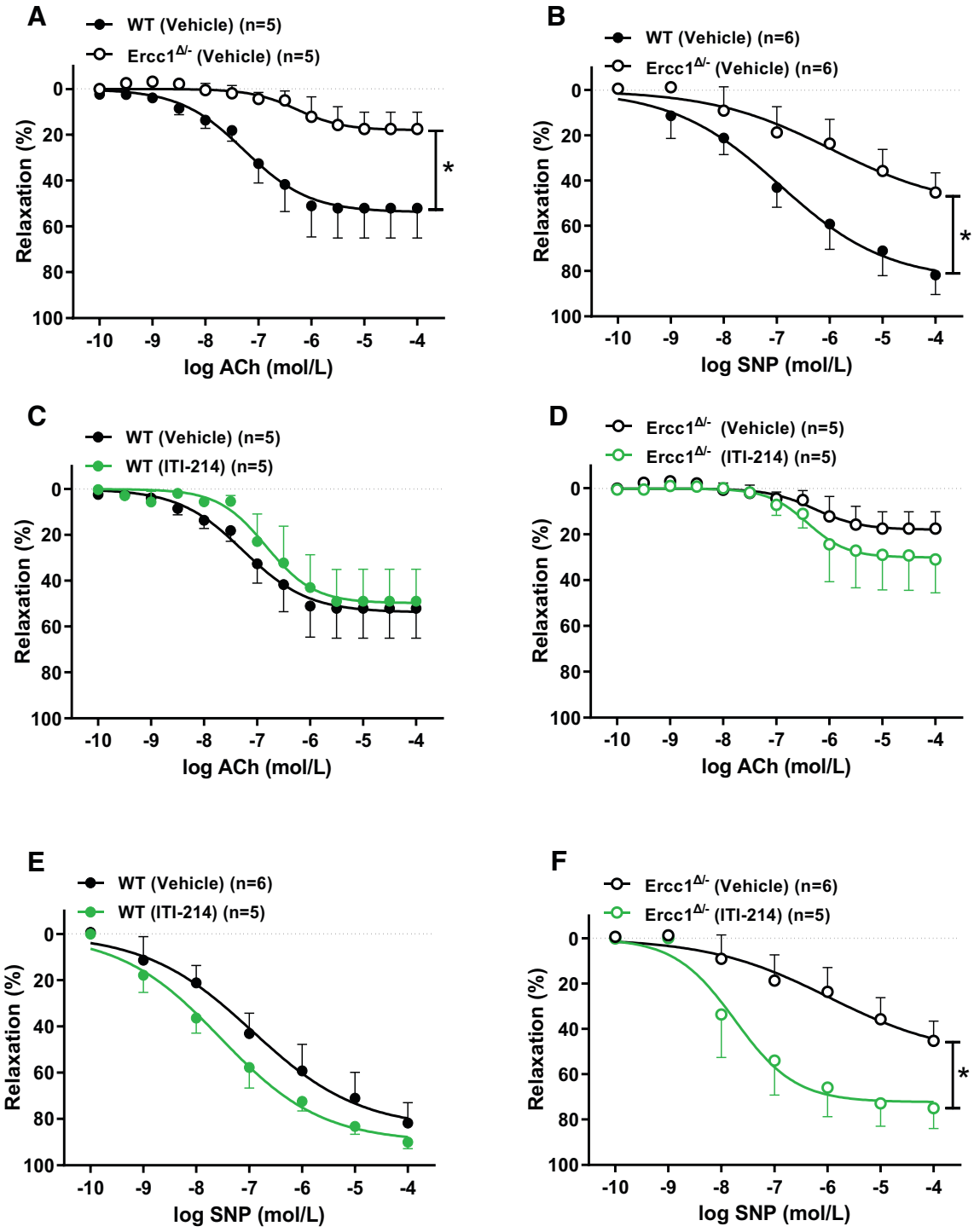

Fig. 5. Endothelium-dependent and -independent responses in isolated coronary rings from *Ercc1*$^{\Delta/-}$ mice (open circles) versus rings from WT littermates (filled circles) measured ex vivo in small wire organ baths (A and B). The endothelium-dependent and -independent effects of 8 weeks of treatment with ITI-214 in drinking water on WT (C and E) and *Ercc1*$^{\Delta/-}$ mice (D and F) (green lines) measured in coronary rings in ex vivo organ bath experiments. Relaxations are calculated relative to the contraction produced by U46619 in each ring, which is set at 100%. Values are expressed as means ± S.E.M.; *$P < 0.05$, GLM for repeated measures.

system (Bautista Niño et al., 2015). In the present study, we explored this hypothesis by investigating the acute and chronic effects of PDE1 inhibition with ITI-214 on main aging features of the macro- and microcirculation and inflammatory status in a mouse model of accelerated aging, *Ercc1*$^{\Delta/-}$, that enables pharmacotherapy during the entire course of the aging process. We show that ITI-214 treatment improves the decreased vasodilation of aorta, coronary artery, and the microcirculation in *Ercc1*$^{\Delta/-}$. In addition, ITI-214 selectively lowered blood pressure and medial hypertrophy in these mice when compared with WT. Interestingly, the treatment reduced the elevated level of proinflammatory cytokines,

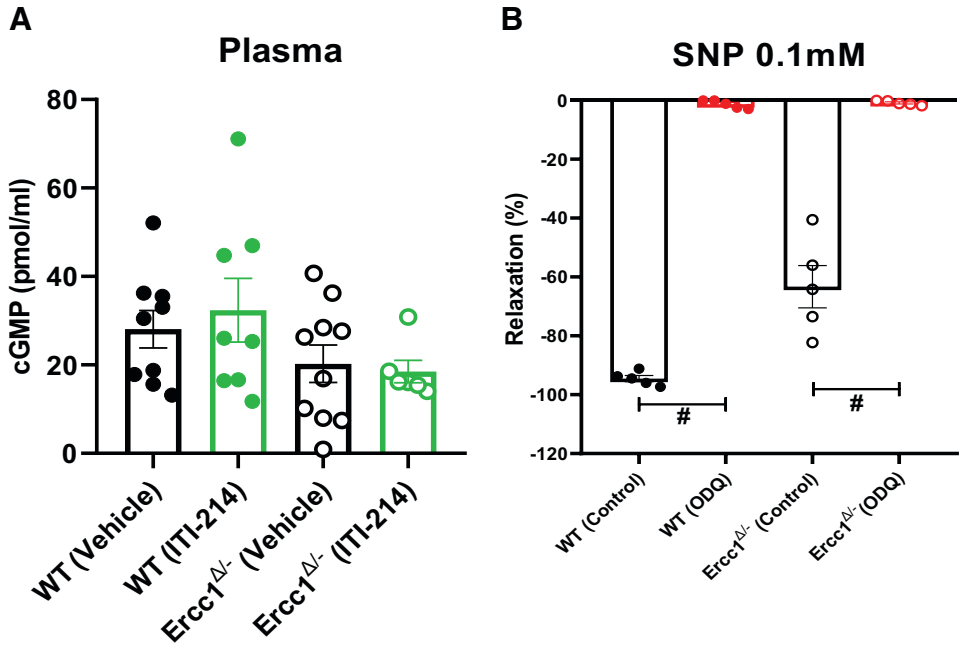

Fig. 6. cGMP level in the plasma (A) and the effect of ODQ on endothelium-independent response (B) in *Ercc1*$^{\Delta/-}$ mice (open circles) versus WT littermates (filled circles). The effects of 8 weeks of treatment with ITI-214 in drinking water are shown in green. Values are expressed as means ± S.E.M.; the number of mice per group is 5–10. Statistical differences were analyzed by two-way ANOVA followed by Bonferroni's post hoc test ($^{\#}P < 0.05$).

whereas anti-inflammatory cytokine levels are preserved. No sex-related differences were observed in any of the measured parameters.

Previously, we tested the possible involvement of PDE1 in disturbed aging-related vasodilation employing the nonselective PDE1/PDE5 inhibitor vinpocetine in aortic rings of *Ercc1*$^{\Delta/-}$ (Bautista Niño et al., 2015). Vinpocetine led to an acute increase in vasodilation, which might have been due to PDE1 inhibition, but might have also involved PDE5. Our present study with ITI-214 demonstrates that acute inhibition of PDE1 alone is sufficient to improve endothelium-independent responses to SNP in the aorta of *Ercc1*$^{\Delta/-}$ mice. The result is in agreement with an earlier study by Khammy et al. (2017) in rat mesenteric arteries, showing that the role of PDE1 is not limited to mice. Notably, the effect of ITI-214 is selective for *Ercc1*$^{\Delta/-}$ and more pronounced in coronary arteries, revealing a potential vessel type–specific effect. The greater effect might be simply explained by the higher degree of dysfunction observed in the coronary artery in *Ercc1*$^{\Delta/-}$ as compared with the aorta. The selectivity of the effect for *Ercc1*$^{\Delta/-}$ might be explained by the fact that SNP responses are already maximal in WT mice. ITI-214 did not improve the responses to ACh, and the correction of response to SNP shows the same pattern (data not shown). This might be explained by the fact that ACh responses in both WT and *Ercc1*$^{\Delta/-}$ are for a large part independent of NO, as was demonstrated by L-NAME. Especially in *Ercc1*$^{\Delta/-}$, in which endothelium-dependent ACh-mediated NO is virtually absent, an acute effect of ITI-214 is not likely to occur. In summary, NO-cGMP signaling is strongly reduced in aged mice and greatly improved by ITI-214. The acute effect of ITI-214 in all likelihood strongly depends on the relative and absolute contribution of NO that can be released by the endothelium during chronological aging or disease conditions.

To our knowledge, this is the first study that explores the consequences of chronic selective PDE1 inhibition on vasomotor function in aging mice, or, for that matter, in any model of vascular disease. The endothelium-dependent and -independent vascular responses were diminished in *Ercc1*$^{\Delta/-}$ mice. The results in the aorta are in line with our previous studies in the same model of accelerated aging. For the first time, we show the vascular dysfunction in the coronary artery of *Ercc1*$^{\Delta/-}$ mice. Chronic ITI-214 improved endothelium-independent vasodilation in the aorta, coronary artery, and the cutaneous microcirculation of *Ercc1*$^{\Delta/-}$ mice, suggesting that PDE1 is relevant for a large array of arteries. In the aorta, we have used a single high dose of SNP after ACh CRC because we know from previous studies that the maximum response to this single high dose of SNP after the ACh CRC duplicates the maximum reached after a full SNP CRC (Golshiri et al., 2020a). Acute administration of ITI-214 to the organ bath yielded no further SNP response in rings of ITI-214–treated mice (data not shown), supporting that ITI-214 was still present in the aortic rings after isolation. The blood pressure was identical in *Ercc1*$^{\Delta/-}$ and WT mice, whereas we have previously shown a modest increase in blood pressure in 16-week-old *Ercc1*$^{\Delta/-}$ mice. This suggests that vasomotor dysfunction might play a role as the early etiological events related to the blood pressure increase. The effect of chronic inhibition of PDE1 in the microcirculation is likely to be related to the blood pressure effect that was observed, as blood pressure regulation predominantly takes place through small resistance arteries. In agreement, both the effect on blood pressure and reactive hyperemia were selective for *Ercc1*$^{\Delta/-}$ as compared with WT. The plasma cGMP levels did not change in treated animals and, considering the tissue-specific distribution of PDE1, we did not expect an effect on plasma cGMP levels. However, since ITI-214 is able to improve SNP responses, and

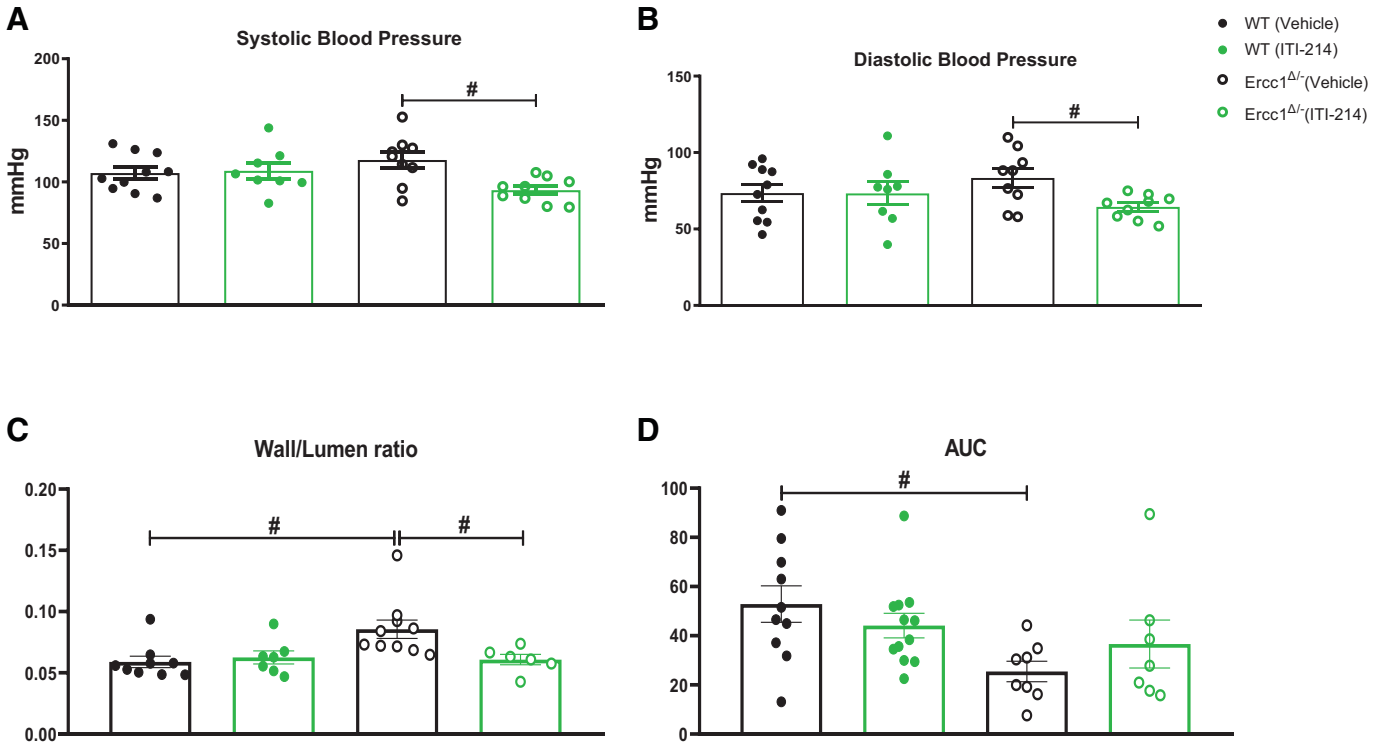

Fig. 7. Systolic and diastolic blood pressure measured by tail-cuff in conscious *Ercc1*$^{\Delta/-}$ mice versus their WT littermates (A and B). The ratio of wall thickness to lumen diameter at 100 mm Hg, as measured by pressure myography technique (C). Cutaneous reactive hyperemia in *Ercc1*$^{\Delta/-}$ mice versus their WT littermates was measured with laser Doppler and expressed as area under the curve (AUC) (D). The number of mice per group is 6–10. Statistical differences were analyzed by two-way ANOVA followed by Bonferroni's post hoc test ($^{\#}P < 0.05$).

the SNP responses can be fully blocked by the sGC inhibitor ODQ, it is evident that ITI-214 is acting through NO/cGMP signaling and improving cGMP responses. Moreover, we found that an alternative hypothesis for improvement of vascular function, blood pressure lowering, can be excluded. The observed improvement in vascular function is independent of blood pressure–lowering effects, as we have previously shown that systolic and diastolic blood pressure lowering by the Ang II type 1 receptor antagonist losartan did not improve vascular aging features in the *Ercc1*$^{\Delta/-}$ model (Wu et al., 2017). Similar to our present study, it was recently shown in hypertensive Dahl salt-sensitive rats on 4% NaCl chow that another selective PDE1 inhibiter (BTTQ) has an antihypertensive effect, both acutely and chronically (Dey et al., 2020). In these rats, a vasodilator response of isolated, preconstricted mesenteric arteries to BTTQ was observed in Dahl SS rats on 0.4% NaCl. Similar to our study, ACh responses were not increased by chronic PDE1 inhibition, which was tested after 21 days in rats fed 4% NaCl. However, the Dahl SS rats in this study did not appear to display endothelial dysfunction since responses reached 90% of the preconstriction with 10 μmol/L phenylephrine. Unfortunately, no low-salt control was included to establish this. Therefore, it remains to be determined what will be the effect of chronic PDE1 inhibition in models with moderately decreased endothelium-dependent relaxations. Nevertheless, PDE1 inhibition may be an option when seeking novel anti-hypertensive treatments, especially in the elderly.

Apart from vasomotor improvements, we also showed the effects of PDE1 chronic inhibition on carotid artery wall hypertrophy. ITI-214 treatment restored the increased wall-to-lumen ratio of *Ercc1*$^{\Delta/-}$ mice to the same ratio as WT. Intimal and medial thickening is attributable to SMC proliferation and migration. Previous studies in cultured VSMC have shown that NO-cGMP decreases proliferation and migration of VSMC, and it is a well known observation that endothelial denudation of the vessel wall or chronic NO inhibition in vivo leads to increased wall thickness (Fingerle et al., 1990; Sarkar et al., 1996; Fischer et al., 2004). In early studies on cardiomyocyte hypertrophy and atherosclerotic plaque formation in the vasculature, PDE1 was identified as a potential drug target to attenuate hypertrophic remodeling (Miller et al., 2009, 2011; Cai et al., 2013). More recent studies in PDE1C-knockout mice confirmed this possibility (Knight et al., 2016). The anti-hypertrophic effect of PDE1 inhibition can relate to both cAMP and cGMP increases, which appear to act independently (Rybalkin et al., 2003). Both cyclic nucleotides control the diameter and wall thickness of arteries because of their effects on vascular relaxation and proliferation. PDE1A and C appear to act separately and specifically on either of these functions depending on the subcellular location of these enzymes. Moreover, species-specific differences have been found, as illustrated elsewhere (Rybalkin et al., 2003). It has also been shown that endogenous cGMP-dependent signaling is able to negatively regulate cardiac hypertrophy by suppressing $G_{q/11}$ activation and normalizing Ca^{2+} signaling (Devynck et al., 2004; Miller et al., 2009). Thus, PDE1 is a very versatile member of the PDE family for the regulation of cardiovascular function.

A major player both in arteriopathy as well as high blood pressure is inflammation (Baudry et al., 1996; Alexander

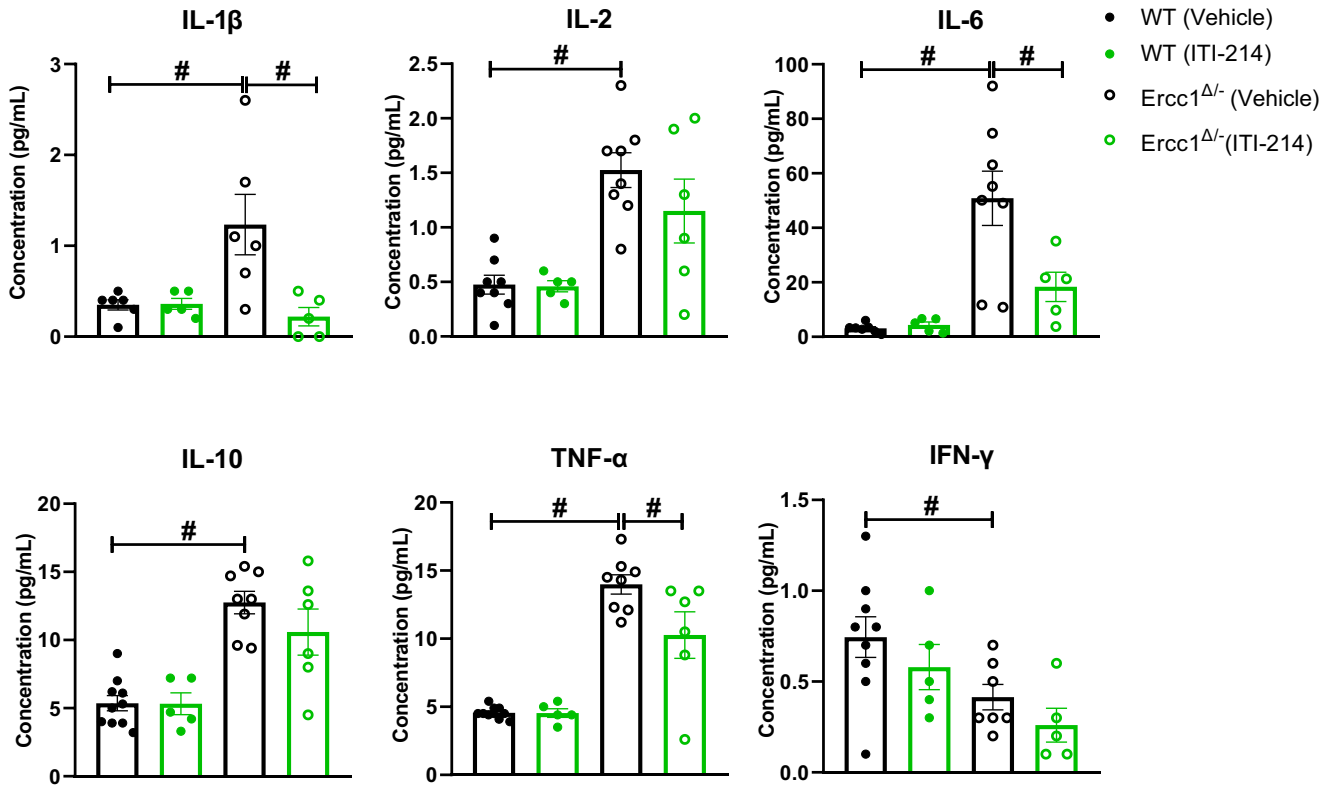

Fig. 8. The concentration of inflammatory cytokines in plasma of *Ercc1*$^{\Delta/-}$ mice and WT littermates. The number of mice per group is 5–10. Statistical differences were analyzed by two-way ANOVA followed by Bonferroni's post hoc test ($^{\#}P < 0.05$).

et al., 2002; Orshal and Khalil, 2004; Granger, 2006; Lee et al., 2006). Proinflammatory cytokines can interact with important blood pressure–regulatory systems, such as the renin-angiotensin system, and also increase the formation of a number of endothelial cell substances, such as endothelin; reduce acetylcholine-induced vasodilatation; and destabilize the mRNA of endothelial nitric oxide synthase, which ultimately affects vascular function and blood pressure regulation (Alexander et al., 2002; Giardina et al., 2002; LaMarca et al., 2005). Hypertrophy can also be stimulated by cytokines such as IL-1β, IL-6, and TNF-α. These cytokines trigger remodeling of the cytoskeleton and change the adhesiveness of the cell to the matrix; additionally, these proinflammatory cytokines are involved in restraining the expression and activity of endothelial nitric oxide synthase; production of oxygen-derived free radicals by neutrophils, VSMCs and ECs; and reduction of NO bioavailability (Sprague and Khalil, 2009; Tian et al., 2016; Chen et al., 2018). We suggest that the reduced level of proinflammatory cytokines by PDE1 inhibition might be an additional anti-hypertrophic mechanism. During aging, a sustained increase in cytokines is associated with vascular dysfunction and vascular disease such as atherosclerosis and hypertension (Peeters et al., 2001; Mirhafez et al., 2014; Moss and Ramji, 2016; Steven et al., 2019; Tyrrell and Goldstein, 2021). We here show that *Ercc1*$^{\Delta/-}$ mice have increased circulating levels of certain cytokines, which is attenuated by PDE1 inhibition. PDE1 inhibition significantly reduced IL-1β, IL-6, and TNF-α proinflammatory cytokines, whereas it did not reduce the elevated level of anti-inflammatory cytokine IL-10. The anti-inflammatory effects of ITI-214 were recently shown by O'Brien et al. (2020) in an in vitro model of an immortalized murine microglial cell line, BV2 cells, possibly by inhibition of ADP-dependent migration via amplified signaling through cAMP and subsequently increased phosphorylation of vasodilator-stimulated phosphoprotein. We now demonstrate the anti-inflammatory properties of PDE1 inhibition in vivo in a mouse model of progressive aging. Thus, apart from vasodilatory and anti-hypertrophic effects through cGMP, PDE1 inhibition also might involve anti-inflammatory effects, which may be cAMP-dependent. It remains to be determined whether ITI-214–mediated reduction in inflammation contributes to augmentation of smooth muscle SNP responsiveness or is an independent event. Moreover, it is currently not known what levels inflammatory factors should reach to have an impact on nonatherosclerotic vascular aging. A complicating observation in this respect is that multiple cytokines are involved, and their temporal-quantitative pattern might be very complex, making it difficult to mimic the conditions in vivo. Thus, it remains unclear whether this statistically significant reduction in proinflammatory cytokines would increase vasodilator capacity. Nevertheless, inflammation, with increased IL-6 and TNF-α as a major mediator, has been implicated in the pathophysiology of hypertension, in particular of end-organ damage (Xiao et al., 2015), and in this light, the present observation that ITI-214 decreases these cytokines is valuable as a future direction when uncovering the mechanisms of treatment effects.

In summary, we demonstrated the role of PDE1 in vasodilation, particularly involving NO-cGMP signaling, and vascular hypertrophy in an accelerated-aging mouse model. We showed

that selective PDE1 inhibition by ITI-214 can attenuate diminished vasodilation and reduced microcirculatory blood flow caused by aging and reduce hypertrophy and the level of proinflammatory cytokines. These findings suggest PDE1 inhibition would have significant therapeutic potential to battle arteriopathy related to aging. It is also worth mentioning that the development of isomer-specific PDE1 inhibitors can shed light on mechanisms involved in PDE1 inhibition and provide more opportunities for future investigations. Application in coronary artery disease might be of specific interest since ITI-214 is currently under development in patients with heart failure to improve cardiac function by enhancing cardiac contractility and dilating systemic arteries without inducing abnormal heart rhythms (identifier: NCT03387215).

Authorship Contributions

Participated in research design: Golshiri, Ataei Ataabadi, Snyder, Davis, Danser, Roks.

Conducted experiments: Golshiri, Ataei Ataabadi, Rubio-Beltran, Dutheil, Yao, Van den Berg-Garrelds, de Vries.

Contributed new reagents or analytic tools: Snyder, van der Pluijm, Brandt, MaassenVanDenBrink.

Performed data analysis: Golshiri, Ataei Ataabadi, Rubio-Beltran, Dutheil, Yao.

Wrote or contributed to the writing of the manuscript: Golshiri, Ataei Ataabadi, Snyder, Davis, Danser, Roks.

References

Alexander BT, Cockrell KL, Massey MB, Bennett WA, and Granger JP (2002) Tumor necrosis factor-alpha-induced hypertension in pregnant rats results in decreased renal neuronal nitric oxide synthase expression. *Am J Hypertens* **15**:170–175.

Ataei Ataabadi E, Golshiri K, Jüttner A, Krenning G, Danser AHJ, and Roks AJM (2020) Nitric oxide-cGMP signaling in hypertension: current and future options for pharmacotherapy. *Hypertension* **76**:1055–1068.

Baudry N, Rasetti C, and Vicaut E (1996) Differences between cytokine effects in the microcirculation of the rat. *Am J Physiol* **271**:H1186–H1192.

Bautista Niño PK, Durik M, Danser AH, de Vries R, Musterd-Bhaggoe UM, Meima ME, Kavousi M, Ghanbari M, Hoeijmakers JH, O'Donnell CJ, et al. (2015) Phosphodiesterase 1 regulation is a key mechanism in vascular aging. *Clin Sci (Lond)* **129**:1061–1075.

Cai Y, Li JD, and Yan C (2013) Vinpocetine attenuates lipid accumulation and atherosclerosis formation. *Biochem Biophys Res Commun* **434**:439–443.

Chen JY, Ye ZX, Wang XF, Chang J, Yang MW, Zhong HH, Hong FF, and Yang SL (2018) Nitric oxide bioavailability dysfunction involves in atherosclerosis. *Biomed Pharmacother* **97**:423–428.

de Almeida AJPO, Ribeiro TP, and de Medeiros IA (2017) Aging: molecular pathways and implications on the cardiovascular system. *Oxid Med Cell Longev* DOI: https://doi.org/10.1155/2017/7941563 [published ahead of print].

Devynck MA, Simon A, Pernollet MG, Chironi G, Gariepy J, Rendu F, and Levenson J (2004) Plasma cGMP and large artery remodeling in asymptomatic men. *Hypertension* **44**:919–923.

Dey AB, Khedr S, Bean J, Porras LL, Meredith TD, Willard FS, Hass JV, Zhou X, Terashvili M, Jesudason CD, et al. (2020) Selective phosphodiesterase 1 inhibitor BTTQ reduces blood pressure in spontaneously hypertensive and dahl salt sensitive rats: role of peripheral vasodilation. *Front Physiol* **11**:543727.

Donato AJ, Machin DR, and Lesniewski LA (2018) Mechanisms of dysfunction in the aging vasculature and role in age-related disease. *Circ Res* **123**:825–848.

Durik M, Kavousi M, van der Pluijm I, Isaacs A, Cheng C, Verdonk K, Loot AE, Oeseburg H, Bhaggoe UM, Leijten F, et al. (2012) Nucleotide excision DNA repair is associated with age-related vascular dysfunction. *Circulation* **126**:468–478.

Fajemiroye JO, da Cunha LC, Saavedra-Rodríguez R, Rodrigues KL, Naves LM, Mourão AA, da Silva EF, Williams NEE, Martins JLR, Sousa RB, et al. (2018) Aging-induced biological changes and cardiovascular diseases. *BioMed Res Int* **2018**:7156435.

Faridounnia M, Folkers GE, and Boelens R (2018) Function and interactions of ERCC1-XPF in DNA damage response. *Molecules* **23**:3205.

Fingerle J, Au YP, Clowes AW, and Reidy MA (1990) Intimal lesion formation in rat carotid arteries after endothelial denudation in absence of medial injury. *Arteriosclerosis* **10**:1082–1087.

Fischer JW, Hawkins S, and Clowes AW (2004) Pharmacologic inhibition of nitric oxide synthases and cyclooxygenases enhances intimal hyperplasia in balloon-injured rat carotid arteries. *J Vasc Surg* **40**:115–122.

Fukuhara S, Sakurai A, Sano H, Yamagishi A, Somekawa S, Takakura N, Saito Y, Kangawa K, and Mochizuki N (2005) Cyclic AMP potentiates vascular endothelial cadherin-mediated cell-cell contact to enhance endothelial barrier function through an Epac-Rap1 signaling pathway. *Mol Cell Biol* **25**:136–146.

Ghebre YT, Yakubov E, Wong WT, Krishnamurthy P, Sayed N, Sikora AG, and Bonnen MD (2016) Vascular aging: implications for cardiovascular disease and therapy. *Transl Med (Sunnyvale)* **6**:183–203.

Giardina JB, Green GM, Cockrell KL, Granger JP, and Khalil RA (2002) TNF-α enhances contraction and inhibits endothelial NO-cGMP relaxation in systemic vessels of pregnant rats. *Am J Physiol Regul Integr Comp Physiol* **283**:R130–R143.

Golshiri K, Ataei Ataabadi E, Brandt R, van der Pluijm I, de Vries R, Danser J, and Roks A (2020a) Chronic sildenafil treatment improves vasomotor function in a mouse model of accelerated aging. *Int J Mol Sci* **21**:1–11.

Golshiri K, Ataei Ataabadi E, Portilla Fernandez EC, Jan Danser AH, and Roks AJM (2020b) The importance of the nitric oxide-cGMP pathway in age-related cardiovascular disease: Focus on phosphodiesterase-1 and soluble guanylate cyclase. *Basic Clin Pharmacol Toxicol* **127**:67–80.

Granger JP (2006) An emerging role for inflammatory cytokines in hypertension. *Am J Physiol Heart Circ Physiol* **290**:H923–H924.

Hashimoto T, Kim GE, Tunin RS, Adesiyun T, Hsu S, Nakagawa R, Zhu G, O'Brien JJ, Hendrick JP, Davis RE, et al. (2018) Acute enhancement of cardiac function by phosphodiesterase type 1 inhibition. *Circulation* **138**:1974–1987.

Insel PA, Murray F, Yokoyama U, Romano S, Yun H, Brown L, Snead A, Lu D, and Aroonsakool N (2012) cAMP and Epac in the regulation of tissue fibrosis. *Br J Pharmacol* **166**:447–456.

Khammy MM, Dalsgaard T, Larsen PH, Christoffersen CT, Clausen D, Rasmussen LK, Folkersen L, Grunnet M, Kehler J, Aalkjaer C, et al. (2017) PDE1A inhibition elicits cGMP-dependent relaxation of rat mesenteric arteries. *Br J Pharmacol* **174**:4186–4198.

Knight WE, Chen S, Zhang Y, Oikawa M, Wu M, Zhou Q, Miller CL, Cai Y, Mickelsen DM, Moravec C, et al. (2016) PDE1C deficiency antagonizes pathological cardiac remodeling and dysfunction. *Proc Natl Acad Sci USA* **113**:E7116–E7125.

LaMarca BB, Cockrell K, Sullivan E, Bennett W, and Granger JP (2005) Role of endothelin in mediating tumor necrosis factor-induced hypertension in pregnant rats. *Hypertension* **46**:82–86.

Lee DL, Sturgis LC, Labazi H, Osborne Jr JB, Fleming C, Pollock JS, Manhiani M, Imig JD, and Brands MW (2006) Angiotensin II hypertension is attenuated in interleukin-6 knockout mice. *Am J Physiol Heart Circ Physiol* **290**:H935–H940.

Li P, Zheng H, Zhao J, Zhang L, Yao W, Zhu H, Beard JD, Ida K, Lane W, Snell G, et al. (2016) Discovery of potent and selective inhibitors of phosphodiesterase 1 for the treatment of cognitive impairment associated with neurodegenerative and neuropsychiatric diseases. *J Med Chem* **59**:1149–1164.

Lugnier C (2006) Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. *Pharmacol Ther* **109**:366–398.

Miller CL, Cai Y, Oikawa M, Thomas T, Dostmann WR, Zaccolo M, Fujiwara K, and Yan C (2011) Cyclic nucleotide phosphodiesterase 1A: a key regulator of cardiac fibroblast activation and extracellular matrix remodeling in the heart. *Basic Res Cardiol* **106**:1023–1039.

Miller CL, Oikawa M, Cai Y, Wojtovich AP, Nagel DJ, Xu X, Xu H, Florio V, Rybalkin SD, Beavo JA, et al. (2009) Role of Ca2+/calmodulin-stimulated cyclic nucleotide phosphodiesterase 1 in mediating cardiomyocyte hypertrophy. *Circ Res* **105**:956–964.

Mirhafez SR, Mohebati M, Feiz Disfani M, Saberi Karimian M, Ebrahimi M, Avan A, Eslami S, Pasdar A, Rooki H, Esmaeili H, et al. (2014) An imbalance in serum concentrations of inflammatory and anti-inflammatory cytokines in hypertension. *J Am Soc Hypertens* **8**:614–623.

Moss JW and Ramji DP (2016) Cytokines: roles in atherosclerosis disease progression and potential therapeutic targets. *Future Med Chem* **8**:1317–1330.

Murray F, Patel HH, Suda RY, Zhang S, Thistlethwaite PA, Yuan JX, and Insel PA (2007) Expression and activity of cAMP phosphodiesterase isoforms in pulmonary artery smooth muscle cells from patients with pulmonary hypertension: role for PDE1. *Am J Physiol Lung Cell Mol Physiol* **292**:L294–L303.

Naka Y, Roy DK, Liao H, Chowdhury NC, Michler RE, Oz MC, and Pinsky DJ (1996) cAMP-mediated vascular protection in an orthotopic rat lung transplant model. Insights into the mechanism of action of prostaglandin E1 to improve lung preservation. *Circ Res* **79**:773–783.

O'Brien JJ, O'Callaghan JP, Miller DB, Chalgeri S, Wennogle LP, Davis RE, Snyder GL, and Hendrick JP (2020) Inhibition of calcium-calmodulin-dependent phosphodiesterase (PDE1) suppresses inflammatory responses. *Mol Cell Neurosci* **102**:103449.

Orshal JM and Khalil RA (2004) Reduced endothelial NO-cGMP-mediated vascular relaxation and hypertension in IL-6-infused pregnant rats. *Hypertension* **43**:434–444.

Oue A, Asashima C, Oizumi R, Ichinose-Kuwahara T, Kondo N, and Inoue Y (2018) Age-related attenuation of conduit artery blood flow response to passive heating differs between the arm and leg. *Eur J Appl Physiol* **118**:2307–2318.

Peeters AC, Netea MG, Janssen MC, Kullberg BJ, Van der Meer JW, and Thien T (2001) Pro-inflammatory cytokines in patients with essential hypertension. *Eur J Clin Invest* **31**:31–36.

Pekcec A, Schülert N, Stierstorfer B, Deiana S, Dorner-Ciossek C, and Rosenbrock H (2018) Targeting the dopamine D_1 receptor or its downstream signalling by inhibiting phosphodiesterase-1 improves cognitive performance. *Br J Pharmacol* **175**:3021–3033.

Rybalkin SD, Yan C, Bornfeldt KE, and Beavo JA (2003) Cyclic GMP phosphodiesterases and regulation of smooth muscle function. *Circ Res* **93**:280–291.

Sarkar R, Meinberg EG, Stanley JC, Gordon D, and Webb RC (1996) Nitric oxide reversibly inhibits the migration of cultured vascular smooth muscle cells. *Circ Res* **78**:225–230.

Snyder GL, Prickaerts J, Wadenberg ML, Zhang L, Zheng H, Yao W, Akkerman S, Zhu H, Hendrick JP, Vanover KE, et al. (2016) Preclinical profile of ITI-214, an inhibitor of phosphodiesterase 1, for enhancement of memory performance in rats. *Psychopharmacology (Berl)* **233**:3113–3124.

Sprague AH and Khalil RA (2009) Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochem Pharmacol* **78**:539–552.
Steven S, Frenis K, Oelze M, Kalinovic S, Kuntic M, Bayo Jimenez MT, Vujacic-Mirski K, Helmstädter J, Kröller-Schön S, Münzel T, et al. (2019) Vascular inflammation and oxidative stress: major triggers for cardiovascular disease. *Oxid Med Cell Longev* **2019**:7092151.
Tian X, Zhao L, Song X, Yan Y, Liu N, Li T, Yan B, and Liu B (2016) HSP27 inhibits homocysteine-induced endothelial apoptosis by modulation of ROS production and mitochondrial caspase-dependent apoptotic pathway. *BioMed Res Int* **2016**:4847874.
Tyrrell DJ and Goldstein DR (2021) Ageing and atherosclerosis: vascular intrinsic and extrinsic factors and potential role of IL-6. *Nat Rev Cardiol* **18**:58–68.
Vermeij WP, Dollé MET, Reiling E, Jaarsma D, Payan-Gomez C, Bombardieri CR, Wu H, Roks AJM, Botter SM, van der Eerden BC, et al. (2016) Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice. *Nature* **537**:427–431.
Versari D, Daghini E, Virdis A, Ghiadoni L, and Taddei S (2009) Endothelial dysfunction as a target for prevention of cardiovascular disease. *Diabetes Care* **32** (Suppl 2):S314–S321.
Weeda G, Donker I, de Wit J, Morreau H, Janssens R, Vissers CJ, Nigg A, van Steeg H, Bootsma D, and Hoeijmakers JHJ (1997) Disruption of mouse ERCC1 results in a novel repair syndrome with growth failure, nuclear abnormalities and senescence. *Curr Biol* **7**:427–439.
Wennogle LP, Hoxie H, Peng Y, and Hendrick JP (2017) Phosphodiesterase 1: a unique drug target for degenerative diseases and cognitive dysfunction. *Adv Neurobiol* **17**:349–384.
Widmer RJ and Lerman A (2014) Endothelial dysfunction and cardiovascular disease. *Glob Cardiol Sci Pract* **2014**:291–308.
Wood RD (2010) Mammalian nucleotide excision repair proteins and interstrand crosslink repair. *Environ Mol Mutagen* **51**:520–526.
Wu H, van Thiel BS, Bautista-Niño PK, Reiling E, Durik M, Leijten FPJ, Ridwan Y, Brandt RMC, van Steeg H, Dollé MET, et al. (2017) Dietary restriction but not angiotensin II type 1 receptor blockade improves DNA damage-related vasodilator dysfunction in rapidly aging *Ercc1Δ/-* mice. *Clin Sci (Lond)* **131**:1941–1953.
Xiao L, Kirabo A, Wu J, Saleh MA, Zhu L, Wang F, Takahashi T, Loperena R, Foss JD, Mernaugh RL, et al. (2015) Renal denervation prevents immune cell activation and renal inflammation in angiotensin II-induced hypertension. *Circ Res* **117**:547–557.

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