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RESEARCH ARTICLE | *Vascular Biology and Microcirculation*

Tobacco smoking induces cardiovascular mitochondrial oxidative stress, promotes endothelial dysfunction, and enhances hypertension

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Dikalov S, Itani H, Richmond B, Arslanbaeva L, Vergeade A, Rahman SM, Boutaud O, Blackwell T, Massion PP, Harrison DG, Dikalova A. Tobacco smoking induces cardiovascular mitochondrial oxidative stress, promotes endothelial dysfunction, and enhances hypertension. *Am J Physiol Heart Circ Physiol* 316: H639–H646, 2019. First published January 4, 2019; doi:10.1152/ajpheart.00595.2018.—Tobacco smoking is a major risk factor for cardiovascular disease and hypertension. It is associated with the oxidative stress and induces metabolic reprogramming, altering mitochondrial function. We hypothesized that cigarette smoke induces cardiovascular mitochondrial oxidative stress, which contributes to endothelial dysfunction and hypertension. To test this hypothesis, we studied whether the scavenging of mitochondrial H_2O_2 in transgenic mice expressing mitochondria-targeted catalase (mCAT) attenuates the development of cigarette smoke/angiotensin II-induced mitochondrial oxidative stress and hypertension compared with wild-type mice. Two weeks of exposure of wild-type mice with cigarette smoke increased systolic blood pressure by 17 mmHg, which was similar to the effect of a subpresssor dose of angiotensin II (0.2 $mg \cdot kg^{-1} \cdot day^{-1}$), leading to a moderate increase to the prehypertensive level. Cigarette smoke exposure and a low dose of angiotensin II cooperatively induced severe hypertension in wild-type mice, but the scavenging of mitochondrial H_2O_2 in mCAT mice completely prevented the development of hypertension. Cigarette smoke and angiotensin II cooperatively induced oxidation of cardiolipin (a specific biomarker of mitochondrial oxidative stress) in wild-type mice, which was abolished in mCAT mice. Cigarette smoke and angiotensin II impaired endothelium-dependent relaxation and induced superoxide overproduction, which was diminished in mCAT mice. To mimic the tobacco smoke exposure, we used cigarette smoke condensate, which induced mitochondrial superoxide overproduction and reduced endothelial nitric oxide (a hallmark of endothelial dysfunction in hypertension). Western blot experiments indicated that tobacco smoke and angiotensin II reduce the mitochondrial deacetylase sirtuin-3 level and cause hyperacetylation of a key mitochondrial antioxidant, SOD2, which promotes mitochondrial oxidative stress.

NEW & NOTEWORTHY This work demonstrates tobacco smoking-induced mitochondrial oxidative stress, which contributes to endothelial dysfunction and development of hypertension. We suggest that the targeting of mitochondrial oxidative stress can be beneficial for treatment of pathological conditions associated with tobacco smoking, such as endothelial dysfunction, hypertension, and cardiovascular diseases.

Listen to this article's corresponding podcast at https://ajpheart.podbean.com/e/mitochondrial-oxidative-stress-in-smoking-and-hypertension/.

catalase; cigarette smoke; hypertension; mitochondria; oxidative stress; superoxide dismutase

INTRODUCTION

Hypertension represents a major risk factor for stroke, myocardial infarction, and heart failure, which cause one-third of deaths worldwide (58, 59). Thirty percent of adults have hypertension (21), and hypertension has been diagnosed in 46% men and 27% women among the Department of Veterans Affairs health care users (41); however, despite treatment with multiple drugs, 37% of patients remain hypertensive (7). Hypertension is a multifactorial disorder (23), and smoking is one of the major risk factors for the development of hypertension (38). Smoking increases blood pressure in both normotensive and hypertensive individuals (22, 46); however, smoking cessation success is very limited (7%) (7a, 8), and the risk for cardiovascular diseases remains elevated long after individuals quit smoking (29a, 35). Furthermore, patients with hypertension who smoke have significantly reduced responses to common classes of antihypertensive drugs, due to metabolic interference between cigarette smoking and drugs (38).

Cigarette smoke exposure results in a dose-dependent inhibition of mitochondrial complex I and complex II, which attenuates mitochondrial respiration and diminishes ATP production (69). Cigarette smoke increases cardiomyocyte ceramide accumulation (67), which increases the production of mitochondrial superoxide ($O_2^{\cdot -}$) and H_2O_2 (19), and alters flow-induced vasodilation (20). Furthermore, cigarette smoke exposure causes metabolic alterations (3) and leads to metabolic reprogramming of the epithelium (54). The deleterious effects of cigarette smoke, however, are not limited to the airway epithelium and spread with circulation to multiple organs, leading to increased central nervous system sympathetic activity (45), endothelial dysfunction (1), and inflammation (42). These harmful effects are mediated by multiple cigarette smoke constituents, such as nicotine and other stable compounds accumulated in cigarette smoke condensate. Although

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the exact mechanism of cigarette smoke-mediated cardiovascular impairment is not fully elucidated, oxidative stress (47) is likely to play an important role in smoking-associated vascular dysfunction and hypertension.

In the past decade, it has become clear that oxidative stress contributes to hypertension by vascular constriction, kidney dysfunction, and inflammation (24). Mitochondria are an important source of $O_2^{\cdot-}$ radicals, and we showed that the mitochondria become dysfunctional in hypertension and defined the novel role of mitochondrial $O_2^{\cdot-}$ in this disease (12, 16). We showed that endothelial dysfunction and hypertension are associated with inactivation of the key mitochondrial antioxidant, SOD2, due to SOD2 hyperacetylation associated with impaired activity of mitochondrial deacetylase sirtuin (Sirt)3 (17). Hypertension is associated with a profound reduction in Sirt3 expression and activity in animal models and humans with essential hypertension (17).

Sirt3 is a key node in the regulation of mitochondrial function (25). It activates mitochondrial metabolism by deacetylation of tricarboxylic acid cycle enzymes (65), complex I (5, 50), and fatty acid β-oxidation enzymes (6, 28), maintains mitochondrial NADPH-GSH redox status by deacetylation of isocitrate dehydrogenase 2 (76), and activates SOD2 by deacetylation of specific lysine residues (66). Interestingly, cigarette smoke promotes both metabolic and redox alterations (4, 51), and nicotine can reduce Sirt3 expression (39). Meanwhile, Sirt3 depletion promotes vascular oxidative stress and hypertension (17).

We hypothesized that cigarette smoke induces mitochondrial oxidative stress, which contributes to endothelial dysfunction and development of hypertension. To test this hypothesis, we examined whether scavenging of mitochondrial H_2O_2 in mitochondria-targeted catalase (mCAT) mice prevents mitochondrial oxidative stress and attenuates hypertension in response to cigarette smoke, investigated the interplay between cigarette smoke and angiotensin II, and defined the potential role of Sirt3 impairment and SOD2 hyperacetylation.

EXPERIMENTAL PROCEDURES

Reagents. The hydroxylamine spin probes 1-hydroxy-4-methoxy-2,2,6,6-tetramethylpiperidine hydrochloride and MitoTEMPO-H were purchased from Enzo Life Sciences (San Diego, CA). DMEM was from ThermoFisher Scientific (Waltham, MA). Sirt3 antibodies (catalog no. D22A3) were obtained from Cell Signaling Technology (Danvers, MA). SOD2 and SOD2-K68 acetyl antibodies (catalog nos. 169560 and 137037) were received from Abcam (San Francisco, CA). All antibodies were validated on knockout and mutant cells. Cigarette smoke condensate was purchased from Murty Pharmaceuticals (Lexington, KY). All other reagents were from Sigma (St. Louis, MO).

Animal experiments. All experimental procedures were approved by the Vanderbilt University Institutional Animal Care and Use Committee. Transgenic mice expressing mCAT and their wild-type littermates (C57BL/6J, The Jackson Laboratory, Bar Harbor, ME) were used. Hypertension was induced by a low-suppressor dose of angiotensin II using osmotic pumps (15) or cigarette smoke exposure (57). Blood pressure was monitored by the tail-cuff method, as previously described (36, 73). Mice (3–4 mo old) were divided into the following four groups: sham, cigarette smoke exposure, angiotensin II infusion, and cigarette smoke exposure plus angiotensin II. Mice were exposed to cigarette smoke for 2 wk using the nose-only exposure setup of the inExpose system (Scireq, Montreal, QC, Canada) by one 10-s puff/min followed by ambient air at 2 l/min for 50 s (4 cigarettes/day). Animals were housed in soft restraints for the duration of cigarette smoke treatment and after treatment were immediately returned to their cages. Sham mice were housed in soft restraints for the duration of cigarette smoke treatment. The potential interplay between the renin-angiotensin system and smoking was tested in cigarette smoke-exposed mice coinfused with a subpressor low dose of angiotensin II (0.2 $mg \cdot kg^{-1} \cdot day^{-1}$), which alone does not cause hypertension (61, 64).

$O_2^{\cdot-}$ measurements by electron spin resonance. Five aortic sections (2 mm) were incubated for 30 min at 37°C in Krebs/HEPES buffer containing 10 μM diethylenetriamene pentaacetate and then placed in a 1-ml syringe and frozen in liquid nitrogen, as previously described (13). Electron spin resonance (ESR) spectra were recorded using the quartz finger Dewar flask. The amplitude of the signal was measured, and the concentration of the detected $O_2^{\cdot-}$ was to be determined by accumulation of the corresponding stable compound nitroxide radical (11), determined from the 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy calibration curve.

Nitric oxide measurements by ESR. Nitric oxide (NO) production in endothelial cells and vessels was quantified by ESR and colloid ferrous-diethyldithiocarbamate 2 [$Fe(DETC)_2$], as we have previously described (9). All ESR samples were placed in a quartz Dewar (Corning, New York, NY) filled with liquid nitrogen. ESR spectra were recorded using an EMX ESR spectrometer (Bruker Biospin, Billerica, MA) and a superhigh-Q microwave cavity. The ESR settings were as follows: field sweep, 160 G; microwave frequency, 9.42 GHz; microwave power, 10 mW; modulation amplitude, 3 G; scan time, 150 ms; time constant, 5.2 s; and receiver gain, 60 dB ($n = 4$ scans).

Vasorelaxation experiments. Isometric tension experiments were performed on 2-mm mouse aortic rings dissected free of perivascular fat. Experiments were performed in a horizontal wire myograph (models 610M and 620M, DMT, Aarhus, Denmark) containing physiological salt solution with the composition of 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose, and 1.8 mM $CaCl_2$. The isometric tone of each vessel was recorded using LabChart Pro v7.3.7 (AD Instruments). Aortic rings were equilibrated over a 2-h period by heating and stretching the vessels to an optimal baseline tension of 36 mN before contracting them with three cycles of 60 mM KCl physiological saline solution. Endothelium-dependent and -independent vascular relaxation was tested after preconstriction with 1 μM phenylephrine. Once the vessels reached steady-state contraction, increasing concentrations of acetylcholine were administered, and the response to each concentration of the drug was recorded.

Measurements of cardiolipin oxidation. Cardiolipin oxidation was measured by liquid chromatography/mass spectrometry (LC/MS), as previously described (75). The extracted lipid fraction was separated online by ultraperformance LC, using a Waters Acquity UPLC system (Waters, Milford, MA). MS analysis was performed on a Quantum Ultra triple-quadrupole mass spectrometer (ThermoFisher Scientific).

Statistics. Experiments were analyzed using a Student's Newman-Keuls post hoc test and ANOVA. P values of <0.05 were considered significant.

RESULTS

Cigarette smoke and angiotensin II cooperatively induced oxidative stress and hypertension. Hypertension is a multifactorial disorder, and exacerbated angiotensin II signaling contributes to the development of endothelial dysfunction and hypertension. We investigated the potential interplay between cigarette smoke and angiotensin II in the development of hypertension. Two-week exposure of wild-type mice to cigarette smoke increased systolic blood pressure by 17 mmHg, which was similar to the effect of a subpressor dose of angiotensin II (0.2 $mg \cdot kg^{-1} \cdot day^{-1}$) (64), leading to a moderate increase to the prehypertensive level (Fig. 1). The combined treatment with cigarette smoke and a low dose of angiotensin II cooperatively induced severe hypertension in wild-type

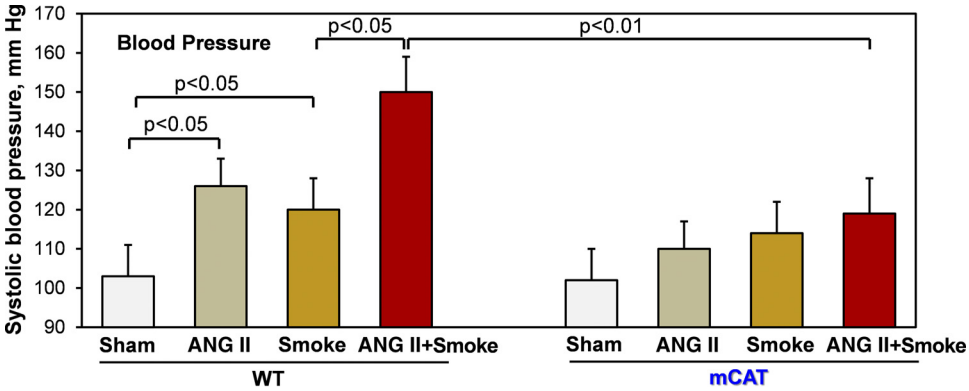

Fig. 1. Cigarette smoke and angiotensin II (ANG II) cooperatively induce hypertension in C57BL/6 mice but not in mice expressing mitochondria-targeted catalase (mCAT). Mice had cigarette smoke nose-only exposure for 2 wk (4 cigarettes/day). Sham mice were housed in soft restraints for the duration of cigarette smoke treatment. Some mice received a subpressor low dose of ANG II (0.2 mg kg^{-1}·day^{-1}), which does not cause hypertension (61, 64). Data are expressed as means ± SD; $n = 6$. P values were calculated by two-way ANOVA. WT, wild type.

mice, whereas the scavenging of mitochondrial H_2O_2 in mCAT mice completely prevented smoke-induced hypertension (Fig. 1). These data strongly support the role of mitochondrial oxidative stress in cigarette smoke-induced hypertension.

The specific role of cigarette smoke exposure in mitochondrial oxidative stress was confirmed by LC/MS analysis of oxidized cardiolipin. Cardiolipin selectively localizes at the matrix side of the mitochondrial inner membrane, and cardiolipin oxidation is a specific marker of mitochondrial oxidative stress (34). Two-week exposure of wild-type mice to cigarette smoke increased cardiolipin oxidation by 30%, whereas angiotensin II infusion doubled cardiolipin oxidation. Interestingly, combined treatment with cigarette smoke and a low dose of angiotensin II cooperatively enhanced cardiolipin oxidation by threefold compared with wild-type sham mice, but the scavenging of mitochondrial H_2O_2 in mCAT mice completely prevented cardiolipin oxidation (Fig. 2).

Cigarette smoke and angiotensin II induced endothelial dysfunction in wild-type but not in mCAT mice. Impaired endothelium-dependent relaxation is a hallmark of endothelial dysfunction in hypertension, due to NO oxidation by $O_2^{\cdot-}$ (37). We tested whether mitochondrial oxidative stress, associated in response to cigarette smoke plus angiotensin II treatment, contributes to impaired vascular relaxation and overproduction of vascular $O_2^{\cdot-}$. We did not see the alteration of endothelium-independent relaxation to the NO donor sodium nitroprusside (data not shown), but cigarette smoke plus angiotensin II led to severe impairment of endothelium-dependent vasorelaxation to acetylcholine, and expression of mitochondria-targeted catalase significantly attenuated the impairment of vasorelaxation (Fig. 3*A*). ESR analysis of aortic sections isolated from wild-type mice exposed to cigarette smoke plus angiotensin II revealed significant $O_2^{\cdot-}$ overproduction (Fig. 3*B*), as measured by ESR and the 1-hydroxy-4-methoxy-2,2,6,6-tetramethylpiperidine hydrochloride spin probe (11). Interestingly, expression of mitochondrial catalase in mCAT mice completely prevented the $O_2^{\cdot-}$ overproduction in cigarette smoke and angiotensin II treatment (Fig. 3*B*).

Cigarette smoke and angiotensin II reduced Sirt3 expression and induced mitochondrial hyperacetylation. Cigarette smoke induces metabolic reprogramming in mitochondria (48, 54), and these metabolic alterations are associated with increased mitochondrial $O_2^{\cdot-}$ and reduced SOD2 activity (43). We have recently shown that hypertension is associated with SOD2 inactivation due to SOD2 hyperacetylation and reduced expression of mitochondrial deacetylase Sirt3 (17). We tested whether cigarette smoke reduces the Sirt3 level and induces SOD2 hyperacetylation, which contribute to hypertension and endothelial dysfunction. To test this hypothesis, we performed Western blot analysis of whole kidneys isolated from mice after 2 wk of treatment with cigarette smoke exposure, angiotensin II infusion, and cigarette smoke exposure plus angiotensin II compared with sham mice (Fig. 4*A*). We previously reported that hypertension is associated with diminished Sirt3 levels both in kidney and vasculature (17), and in this work, we analyzed Sirt3 expression in the kidney, due to the tissue availability. It was found that cigarette smoke exposure and angiotensin II infusion induced SOD2 hyperacetylation (Fig. 4*B*), which is linked to diminished SOD2 activity (17). Interestingly, SOD2 expression was not changed significantly (Fig. 4*C*). SOD2 acetylation was accompanied by a moderate reduction of the mitochondrial deacetylase Sirt3 level (Fig. 4*D*).

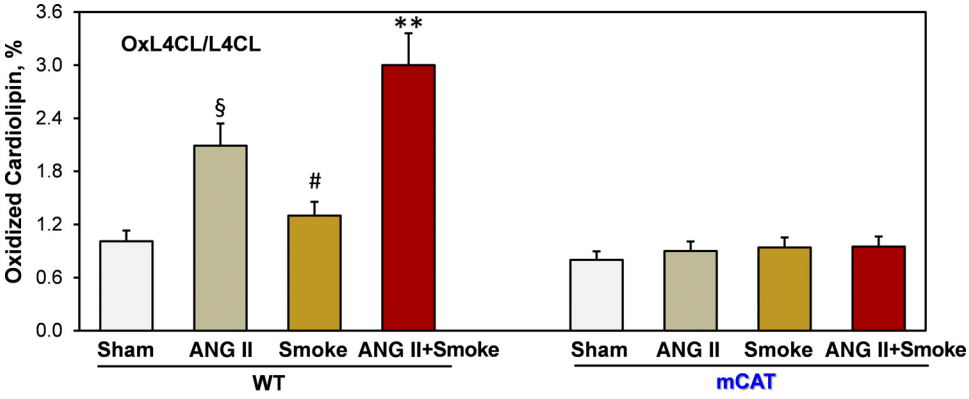

Fig. 2. Cigarette smoke and angiotensin II (ANG II) cooperatively induce oxidation of mitochondrial cardiolipin in C57BL/6 mice but not in mice expressing mitochondria-targeted catalase (mCAT). Mice had cigarette smoke nose-only exposure (4 cigarettes/day), a subpressor low dose of ANG II infusion (0.2 mg·kg^{-1}·day^{-1}), or combined ANG II + smoke treatment. After 2 wk of treatment, mice were euthanized for liquid chromatography/mass spectrometry analysis of oxidized cardiolipin in the heart. Data are expressed as means ± SD; $n = 6$. §$P < 0.01$ vs. sham mice; #$P < 0.05$ vs. sham mice; **$P < 0.01$ vs. ANG II. L4CL, tetralinoleoylcardiolipin; oxL4CL, monohydroxy L4CL; WT, wild type.

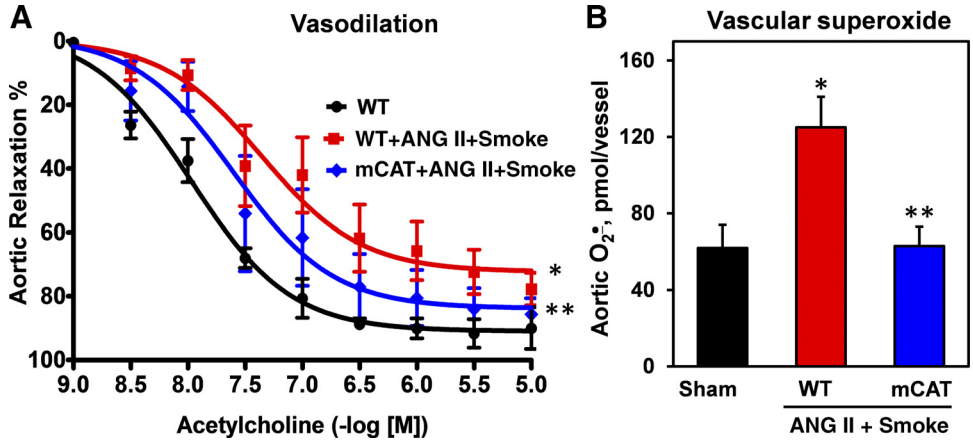

Fig. 3. Vasodilatation (*A*) and vascular superoxide ($O_2^{\cdot-}$) production (*B*) in aortas isolated from mice treated with a combination of cigarette smoke and angiotensin II (ANG II; 0.2 mg·kg^{-1}·day^{-1}) for 2 wk. Vascular $O_2^{\cdot-}$ was measured by the 1-hydroxy-4-methoxy-2,2,6,6-tetramethylpiperidine hydrochloride spin probe and electron spin resonance, as we have previously described (11). Results are means ± SD; $n = 6$. *$P < 0.01$ vs. wild-type (WT) mice; **$P < 0.05$ vs. WT + ANG II/smoke. mCAT, mice expressing mitochondria-targeted catalase.

Of note, the extent of SOD2 hyperacetylation does not match the loss of Sirt3 expression. For example, combined cigarette smoke exposure plus angiotensin II infusion reduced Sirt3 by 43% and increased SOD2 acetylation by 3.5-fold. These data suggest a potential role of increased acetylation rate and redox Sirt3 inactivation (17) in the imbalance of SOD2 acetylation-deacetylation, leading to SOD2 hyperacetylation in response to cigarette smoke.

Endothelial dysfunction induced by cigarette smoke condensate. Our data show that cigarette smoke induces SOD2 hyperacetylation and mitochondrial oxidative stress. This can be mediated by increased mitochondrial $O_2^{\cdot-}$, which oxidizes and inactivates endothelial NO, leading to impaired vascular relaxation. To test this hypothesis, we studied whether cigarette smoke can directly affect vascular function. It was found that incubation of mouse aortic sections with cigarette smoke condensate increases the production of mitochondrial $O_2^{\cdot-}$ by 50% and reduces the endothelial NO by 51%, as measured by the mitochondria-targeted spin probe MitoTEMPO-H (11) and specific NO spin trap $Fe(DETC)_2$ (9, 10) by ESR (Fig. 5). These data support that cigarette smoke alters mitochondrial function, leading to an imbalance of mitochondrial SOD2 acetylation and increased $O_2^{\cdot-}$ production, which contributes to endothelial oxidative stress and hypertension.

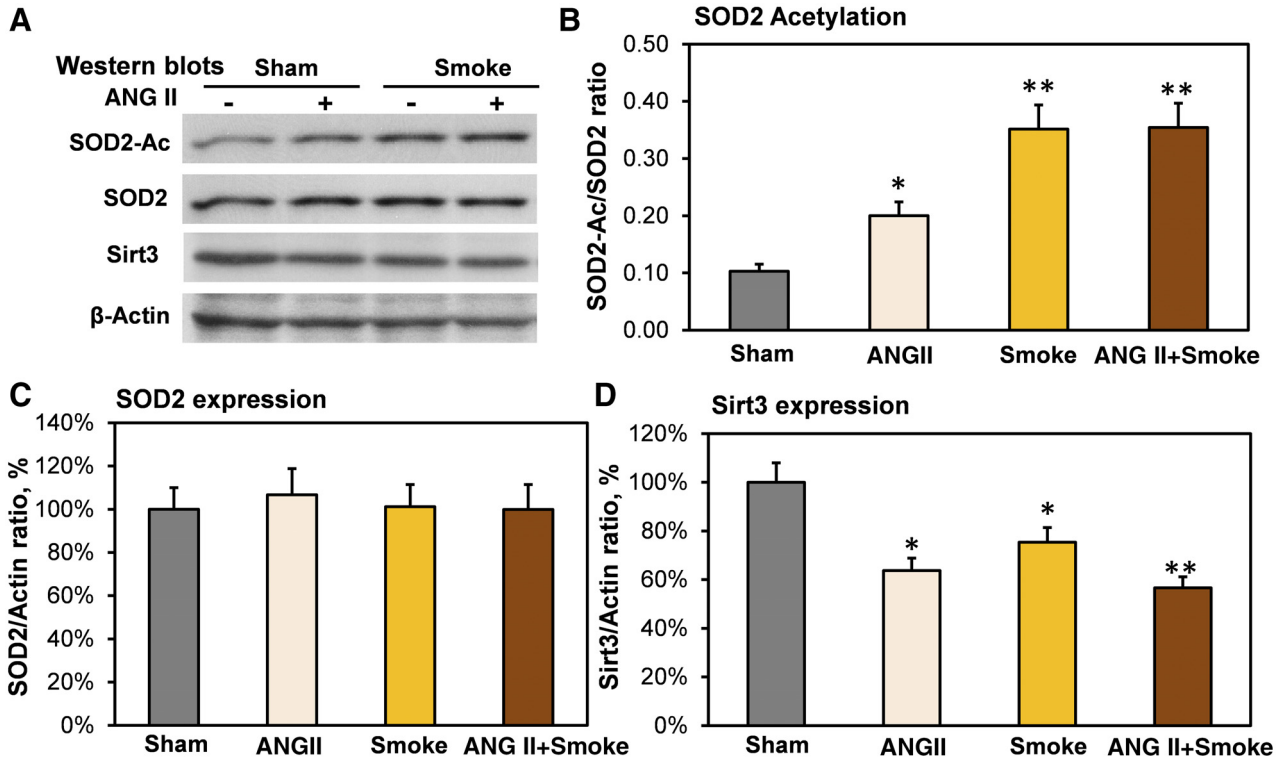

Fig. 4. Effect of cigarette smoke and angiotensin II (ANG II) on acetylation of mitochondrial antioxidant SOD2 (SOD2-Ac), expression of SOD2, and sirtuin 3 (Sirt3). Mice had cigarette smoke exposure, ANG II infusion, or combined ANG II + smoke treatment. After 2 wk of treatment, mice were euthanized for kidney Western blot experiments (*A*). Cigarette smoke exposure and ANG II infusion induced SOD2 hyperacetylation (*B*), which diminished SOD2 activity (17). SOD2 expression was not significantly changed (*C*). SOD2 acetylation was accompanied by a moderate reduction of mitochondrial deacetylase sirtuin 3 (Sirt3; *D*). Data are normalized by β-actin level and expressed as means ± SE; $n = 6$. *$P < 0.01$ vs. sham mice; **$P < 0.001$ vs. ANG II.

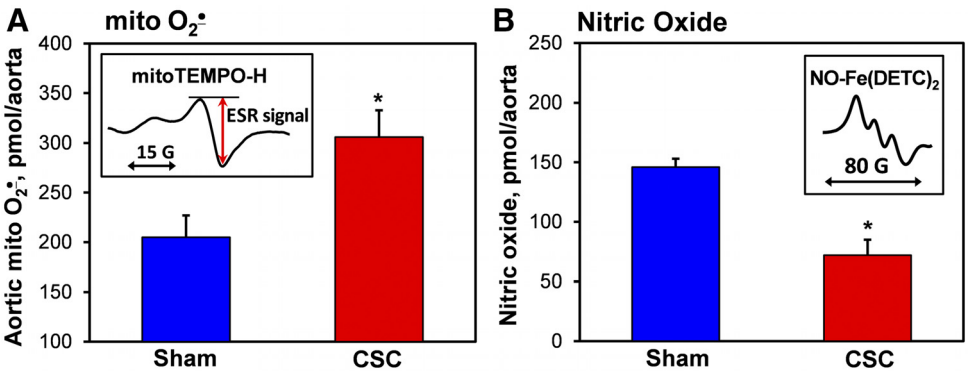

Fig. 5. Effect of cigarette smoke condensate (CSC) on mitochondrial superoxide (mito $O_2^{\cdot-}$) production (*A*) and endothelial nitric oxide (NO; *B*) in the mouse aorta. Aortic sections isolated from sham wild-type mice were treated in DMEM tissue culture medium with CSC (40 μg/ml) or DMSO as vehicle (sham) for 24 h before electron spin resonance (ESR) measurements (*insets*) (10). *$P < 0.01$ vs. sham mice ($n = 6$). $Fe(DETC)_2$, ferrous-diethyldithiocarbamate.

DISCUSSION

This study provides the first evidence that cigarette smoke induces SOD2 hyperacetylation and enhances endothelial dysfunction and, cooperatively with angiotensin II, induces mitochondrial oxidative stress, which promotes the development of hypertension. Our data indicate that acute cigarette smoke exposure for 2 wk reduces expression of mitochondrial deacetylase Sirt3 and increases SOD2 hyperacetylation, which increases mitochondrial $O_2^{\cdot-}$, leads to cardiolipin oxidation, and contributes to impaired vascular relaxation (Figs. 2–5). It is interesting that the scavenging of mitochondrial H_2O_2 in transgenic mice expressing mitochondria-targeted catalase completely prevents overproduction of mitochondrial $O_2^{\cdot-}$ and inhibits cigarette smoke-induced oxidative stress and hypertension (Figs. 1 and 2).

Tobacco smoking is strongly associated with oxidative stress (47). Clinical studies have shown that accumulation of the lipid peroxidation product malondialdehyde in blood plasma of smokers was increased by 2.5-fold, whereas activity of the major antioxidant enzymes catalase, SOD, and glutathione peroxidase was reduced significantly (44). This leads to oxidation of cysteine and glutathione, and the level of reduced glutathione is diminished in the kidney by twofold in mice exposed to cigarette smoke for 4 days (56). The resultant alteration in the thiol redox status impairs cellular redox signaling and can cause cellular dysfunction. Indeed, the smoking of a single cigarette rapidly reduces endothelial NO production and significantly diminishes blood plasma antioxidants (68). It has been proposed that cigarette smoke induces $O_2^{\cdot-}$ production in endothelial cells, leading to NO inactivation and NO synthase (NOS) uncoupling (53). Treatment of cultured endothelial cells with cigarette smoke condensate or smokers' blood plasma increases cellular $O_2^{\cdot-}$ production and reduces NOS activity (31, 49). The specific mechanisms of smoking-mediated endothelial dysfunction and hypertension, however, remain unclear. Our data demonstrate that cigarette smoke directly induces mitochondrial $O_2^{\cdot-}$ and diminishes endothelial NO, which promote hypertension.

We have previously shown that cigarette smoke condensate induces metabolic reprogramming in mitochondria, which contributes to cancer development (48, 54). These metabolic alterations are associated with reduced SOD2 activity (43) and increased NADPH/NADH accumulation (54). The increased NADPH level can partially compensate for the mitochondrial oxidative stress, due to NADPH-dependent maintenance of the thiol redox status by glutathione reductase and thioredoxin reductase (32) as well as the scavenging of H_2O_2 and lipid peroxide by glutathione peroxidases (63). Our data show increased cardiolipin oxidation and blood pressure in wild-type mice exposed to cigarette smoke, which was prevented by the mitochondrial catalase, indicating that cigarette smoke-induced metabolic reprogramming does not completely compensate for the mitochondrial oxidative stress.

Our data indicate that expression of mitochondria-targeted catalase protects from cigarette smoke-induced mitochondrial oxidative stress measured by cardiolipin oxidation and attenuates endothelial dysfunction and hypertension (Figs. 1–3). Meanwhile, mCAT mice do not induce mitochondrial $O_2^{\cdot-}$ overproduction (30), and these mice have higher SOD2 activity (17), due to SOD2 deacetylation compared with wild-type littermates. This provides mCAT mice with enhanced activities of catalase and SOD2 in mitochondria, which protect mitochondria from both $O_2^{\cdot-}$ and H_2O_2. These data, therefore, do not provide definitive information as to whether $O_2^{\cdot-}$ or H_2O_2 is critical in the mediation of the effect of smoking and angiotensin II on blood pressure. We have previously shown that SOD2-overexpressing mice are protected from hypertension and endothelial dysfunction; however, mCAT mice have normal SOD2 expression, similar to wild-type mice. The scavenging of mitochondrial H_2O_2 is important in redox homeostasis of mitochondria (63), and it improves Sirt3-mediated SOD2 deacetylation (17). Indeed, the maintenance of thiol

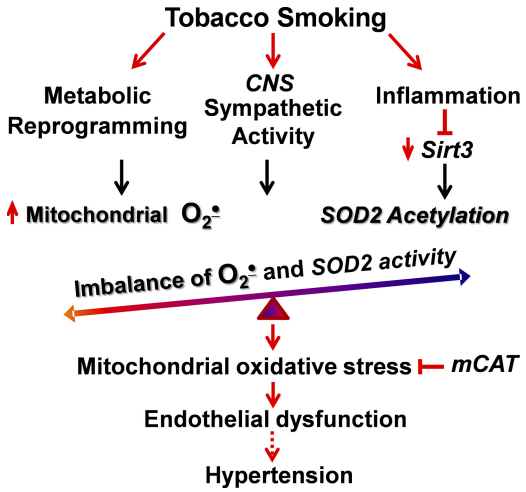

Fig. 6. Proposed role of mitochondrial oxidative stress in tobacco smoking-mediated endothelial dysfunction and hypertension. CNS, central nervous system; mCAT, mitochondria-targeted catalase; $O_2^{\cdot-}$, superoxide; Sirt3, sirtuin 3.

redox balance plays a key role in the regulation of mitochondrial function, and disruption of this redox organization is a common basis for disease (32, 33). Sirt3 is a key node in the regulation of mitochondrial function (25), and we have recently shown that Sirt3 *S*-glutathionylation contributes to Sirt3 inactivation in hypertension (17). The scavenging of mitochondrial H_2O_2 in transgenic mCAT mice prevents Sirt3 *S*-glutathionylation, reduces SOD2 hyperacetylation, and attenuates angiotensin II-induced hypertension (17), whereas treatment with the mitochondria-targeted H_2O_2 scavenger MitoEbselen, after the onset of hypertension, improved Sirt3 deacetylase activity and reduced blood pressure in wild-type mice (17). Furthermore, mitochondrial H_2O_2 plays an important role in redox cell signaling and contributes to ROS-induced ROS production by NADPH oxidases, xanthine oxidase, and other sources (12, 78). It is conceivable that Sirt3 redox inactivation and redox-dependent stimulation of ROS production contribute to cigarette smoke-induced mitochondrial alterations and endothelial dysfunction.

The diminished endothelial NO level is a hallmark of endothelial dysfunction in hypertension, and our data show that cigarette smoke and cigarette smoke condensate reduce NO and impair endothelial-dependent relaxation (Figs. 2 and 4). This can be associated with a diminished NOS activity and/or NO oxidation by $O_2^{\cdot-}$. Indeed, cigarette smoke increases $O_2^{\cdot-}$ production, which contributes to impairment of endothelial-dependent relaxation corrected by SOD (62), whereas endothelial-independent relaxation is preserved in acute cigarette smoke exposure (18), suggesting that cigarette smoke-induced $O_2^{\cdot-}$ in endothelium contributes to a reduction of vascular NO levels. The precise mechanism of a smoke-induced reduction of NO, however, remains unclear. It can include the uncoupling and inhibition of NOS. L-Arginine depletion or $O_2^{\cdot-}$/peroxynitrite-mediated tetrahydrobiopterin oxidation results in the uncoupling of NOS, leading to $O_2^{\cdot-}$ production rather than NO release (70). Indeed, cigarette smoke extract increases $O_2^{\cdot-}$ production and depletes the essential NOS cofactor tetrahydrobiopterin (1), and tetrahydrobiopterin supplementation improves endothelium-dependent relaxation in chronic smokers (26). Furthermore, supplementation L-arginine improves endothelium-dependent relaxation in a smoke exposure model (29). Cigarette smoke decreases expression of arginine transporter cationic amino acid transporter 1 and increases accumulation of an endogenous inhibitor of endothelial NOS, asymmetric dimethylarginine (77). It is conceivable that metabolic reprogramming and oxidative stress, in response to tobacco smoking, contribute to NO inactivation, NOS inhibition, and uncoupling. Further studies are warranted to define potential supplements that can improve the endothelium-independent relaxation in individuals with a history of tobacco smoking.

Endothelial dysfunction is critically contributing to the development of hypertension, which is associated with impaired relaxation in both resistance and conductance vessels (72). In this work, we studied the effect of tobacco smoking on oxidative stress and impaired endothelial-dependent relaxation in the aorta; however, blood pressure is regulated by microcirculation in the resistance vessels, such as mesenteric arteries (40). In patients with hypertension, relaxation of resistant arteries to acetylcholine is blunted, and it is not affected by NOS inhibition but is improved by the NADPH inhibitor apocynin (71), indicating an impaired NO pathway in patients with hypertension and the potential role of oxidative stress. Endothelium-dependent relaxation of both the aorta and mesenteric arteries is redox dependent (27, 74); however, the lack of NO-dependent relaxation in resistance vessels can be partially compensated by NOS-independent relaxation (60). Endothelial dysfunction in both resistance and conductance vessels contributes to the end-organ damage in hypertension; however, additional studies are required to establish the specific effect of tobacco smoking on the function of resistance vessels, such as mesenteric arteries.

Hypertension is a multifactorial phenomenon that is mediated by a series of central, inflammatory, and metabolic pathways (23). The cross-talk among these multiple pathways increases oxidative stress in multiple organs, and ROS is critical in the pathophysiology of hypertension (14). Interestingly, tobacco smoking increases inflammation (42), stimulates central nervous system sympathetic activity (45), and causes metabolic alterations (54), which are all important risk factors of endothelial dysfunction and hypertension. We propose that tobacco smoking, by acting on these multiple pathways, leads to the development of mitochondrial oxidative stress, which contributes to the development of hypertension (Fig. 6). Indeed, in this work, we found that cigarette smoke exposure promotes mitochondrial dysfunction by Sirt3 depletion, SOD2 hyperacetylation, and cardiolipin oxidation. It is conceivable that mitochondria-targeted interventions may correct the metabolic, central, vascular, and inflammatory alterations that contribute to the increased risk for cardiovascular diseases, even long after individuals quit smoking (29a, 35).

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.D. conceived and designed research; S.D., H.I., B.R., L.A., A.V., and A.D. performed experiments; S.D., H.I., B.R., L.A., A.V., and A.D. analyzed data; S.D., L.A., and A.D. interpreted results of experiments; S.D., L.A., and A.D. prepared figures; A.D. drafted manuscript; S.D., S.M.J.R., O.B., T.B., P.P.M., D.G.H., and A.D. edited and revised manuscript; S.D., H.I., B.R., L.A., A.V., S.M.J.R., O.B., T.B., P.P.M., D.G.H., and A.D. approved final version of manuscript.

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