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Contribution of arginase activation to vascular dysfunction in cigarette smoking

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ABSTRACT

Background: Cigarette smoke increases the risk of several cardiovascular diseases and has synergistic detrimental effects when present with other risks that contribute to its pathogenesis. Oxidative injury to the endothelium via reactive oxygen species (ROS) and nitric oxide (NO) dysregulation is a common denominator of smoking-induced alterations in vascular function. However, the mechanisms underlying ROS and NO dysregulation due to smoking remain unclear. We tested if arginase (Arg) activation/upregulation contributes to this phenomenon by constraining nitric oxide synthase (NOS) activity.
Methods: Arg2 knockout ($Arg2^{-/-}$) and control C57BL/6J mice were either exposed to cigarette smoke, 6 h/day/2 weeks (Second Hand Smoking; SHS) or housed in normal environment (Non Smoking; NS). Arg activity, NO and ROS levels were determined by measuring urea production, fluorescent dye (DAF), and dihydroethedium (DHE) respectively in isolated mouse aorta.
Results: Arg activity and ROS levels were higher NO lower in SHS compared to NS mice. SHS failed to lower NO levels in $Arg2^{-/-}$ mice. Endothelial dependent vasodilation (EDV) was attenuated in SHS mice as compared to controls (78.80% ± 8 vs 46.58% ± 5). This impaired EDV was abolished in $Arg2^{-/-}$ mice (67.48% ± 7 in SHS vs. 78.80% ± 8 in NS). Vascular stiffness was increased in SHS mice as compared to NS controls but remained unchanged in $Arg2^{-/-}$ mice.
Conclusion: Endothelial NOS is uncoupled by smoking exposure, leading to endothelial dysfunction and vascular stiffness, a process that is prevented by Arg2 deletion. Hence, we identify Arg2 upregulation as a critical pathogenic factor and target for therapy in oxidative injury following smoking exposure through reciprocal regulation of endothelial NOS.

1. Introduction

Cigarette Smoking is a major risk factor for atherosclerotic cardiovascular disease (CVD) including coronary heart disease and accounts for one in every six deaths in the United States [1]. Furthermore, smokers are two times as likely to suffer from a stroke [2], [3].

Endothelial dysfunction is an early and critical event that occurs in the development of CVD [4]. In addition to complex genetic and environmental factors, cigarette smoke plays a pivotal role in the pathogenesis of vascular endothelial dysfunction and the resultant comorbidities [5]. While the mechanisms of endothelial dysfunction are not fully understood, decreased nitric oxide (NO) production and increased ROS are well-documented consequences of cigarette smoke exposure in animal models [6]. Reduced NO production is known to impair vasodilation, one of the key mechanisms underlying the pathogenesis of endothelial dysfunction and CVD [5]. In cultured endothelial cells, cigarette smoke extract has been shown to result in decreased NO bioavailability. There is conflicting evidence regarding regulation of endothelial NOS (eNOS) by cigarette smoke extract at the transcriptional/translational level in cellular models with some suggesting reduced eNOS mRNA/protein levels [5,7] while others demonstrating unchanged levels [8]. Cigarette smoke has also been shown to inhibit eNOS-dependent NO production by altering eNOS phosphorylation favoring the uncoupled monomer state over the

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dimer state [9]. Furthermore, human umbilical vein endothelial cells treated with serum from smokers demonstrated higher eNOS expression but lower eNOS activity (NO production) compared to cells treated with serum from non-smokers [10].

Arginase reciprocally regulates eNOS activity by competing for the common substrate, L-arginine, in various pathologies including erectile dysfunction, heart failure and atherogenesis [11–13]. Arg1 and Arg2 are two distinct Arg isoforms encoded by different genes [14]. Arg1 is manly intrahepatic and its expression can be induced by numerous pro-inflammatory factors like LPS, TNFα, hypoxia, interferon γ, 8-bromo-cGMP and oxidized-LDL [15–21]. Arg2 is extra-hepatic isoform and is also the principal form present in the vascular endothelium [22]. In the present study we test the hypothesis that Arg2 activation/upregulation contributes to endothelial dysfunction caused by cigarette smoke by constraining eNOS-dependent NO production.

2. Material and methods

2.1. Animal model

Wild type (C57BL/6J, WT) and Arginase 2 Knockout ($Arg2^{-/-}$) male mice (3–4 months old, $n = 6$) were exposed to 2 weeks of cigarette smoke with their control counterparts exposed to room air [2]. Cigarette smoke was delivered to WT and $Arg2^{-/-}$ mice for 6 h per day during 5 days per week over a period of 2 weeks by burning 2R4F reference cigarettes (Tobacco Research Institute, University of Kentucky) using a TE-10 smoking machine (Teague Enterprises, CA; Control). Air exposed control mice were housed in a filtered air environment (Non Smoking; NS).

2.2. Pulse wave velocity

Aortic stiffness, an index of vascular health in vivo, was assessed by measuring pulse wave velocity (PWV) using a high frequency, high resolution doppler spectrum analyzer (DSPW), a real-time signal acquisition and spectrum analyzer system (Indus Instruments, Houston, Texas) as described earlier [23]. Mice were lightly anesthetized with 1.5% isoflurane, blood pressures and heart rates were allowed to stabilize into the physiologic range prior to study. 10 and 20 MHz probes were used to measure the descending aortic and abdominal aortic flow velocity.

2.3. Nitric oxide and reactive oxygen species

Nitric oxide (NO) was measured using either NO-sensitive fluorescent dye (DAF-FM) or using Griess Reagent as described previously [24,25]. Reactive oxygen species (ROS) were measured using DHE [12]. For both fluorescent dyes, recordings were made using upright fluorescent microscope (Nikon Eclipse 80i) in en-face endothelial side up, probe labeled aortic tissue. Fluorescent data was analyzed using NIS-ELEMENTS Basic Research.

2.4. Endothelial function

Thoracic aortas were isolated, trimmed into 2-mm rings, and mounted in organ baths containing 37 °C oxygenated Krebs solution. Isometric force recording was conducted using a DMT myograph by Danish Myo Technology A/S Aarhus, Denmark as described earlier [26]. Stretched vessels were constricted with 1 μM phenylephrine in the presence of 3 μM Indomethacin. A dose–response curve was then obtained using 10^{-9}–10^{-5} M acetylcholine.

2.5. Arginase activity

Arginase activity was measured by assaying urea using a colorimetric method with–isonitrosopropiophenone as described previously [27].

2.6. Statistics

All statistical analyses were performed using Prism 5 for Mac by GraphPad Software Inc. and Microsoft Excel version 14.1.3 statistical analysis software. The results were expressed as mean and standard error (mean ± SEM). One-way analysis of ANOVA and the Bonferroni post hoc test for multiple-comparison were used for comparing all groups and pairs of groups respectively. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Second hand smoke increases arginase activity and uncouples eNOS

Aortas isolated from WT mice that were exposed to cigarette smoke exhibited a significant upregulation in arginase activity (2.5 fold as compared to NS controls; WT Control vs. WT SHS; 454.66 ± 56 vs. 195.33 ± 14 AU, $p \leq 0.001$, Fig. 1A). There was a significant attenuation in NO production in WT mice exposed to cigarette smoke (WT SHS vs. WT Control; 0.005 vs. 0.013, $p \leq 0.05$, $n = 4$) which was absent in $Arg2^{-/-}$ mice that were exposed to the same level of cigarette smoke ($Agr2^{-/-}$ SHS vs. $Agr2^{-/-}$ Control; 0.013 vs. 0.012, $p > 0.05$, $n = 4$, Fig. 1C). Aortas from WT mice exposed to cigarette smoke also had higher levels of ROS (WT SHS vs. WT Control; 446.00 ± 49.00 vs. 148.30 ± 6.67AU, $p \leq 0.001$, Fig. 1B).

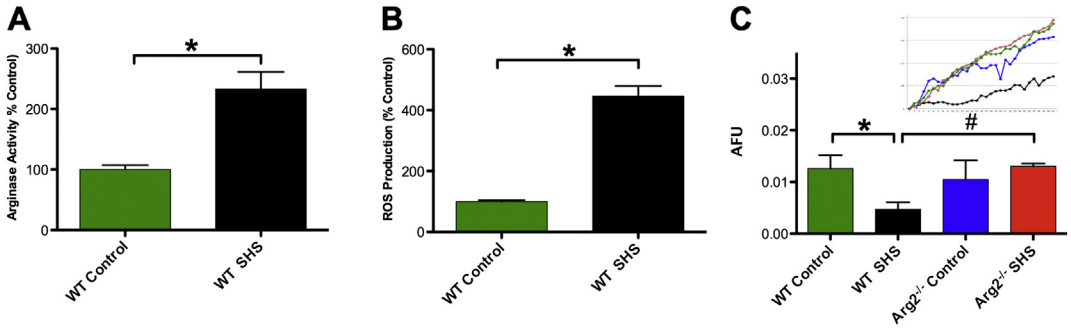

Fig. 1. Second hand cigarettes exposure leads to increased total arginase activity and eNOS uncoupling. A) Total arginase activity increased significantly in animals exposed to cigarette smoke B) reactive oxygen species (ROS) were significantly higher animals exposed to cigarette smoke C) Endothelial NO production was significantly lower in WT animals exposed to cigarette smoke, but was did not differ in arginase KO animals. Inlet: Rate of NO and ROS production was calculated as the slope of the fluorescence measured over time was significantly lower in animals exposed to cigarette smoke. *$p < 0.05$ WT SHS vs. WT Control, #$p < 0.05$ WT SHS vs. $Arg2^{-/-}$ SHS.