Atherosclerosis: Open Access

Han, Atheroscler Open Access 2022, 7:6

Review Article Open Access

Atherosclerosis by Targeting the Mitochondria-Inflammation Circle

Jihong Han*

Key Laboratory of Metabolism and Regulation for Major Diseases of Anhui Higher Education Institutes, Hefei University of Technology, Hefei, 230009, China

Abstract

The mitochondrial redox equilibrium of endothelial cells (ECs) may become disturbed, which may result in persistent inflammation and atherosclerosis. Oxidative damage can cause endothelial dysfunction, and chronic sympathetic hyperactivity can make it worse. By reducing mitochondrial reactive oxygen species (ROS)-induced inflammation, we investigated whether renal denervation (RDN), a method for lowering sympathetic tone, could protect ECs from atherosclerosis.

ApoE-deficient (ApoE/-) mice underwent RDN or a sham procedure prior to consuming a high-fat diet for 20 weeks. The mitochondrial morphology, atherosclerosis, and EC phenotype were all found. Norepinephrine treatment of human artery ECs was used in vitro to investigate the underlying mechanisms of RDN-repressed endothelial inflammation. In EC mitochondria, RDN reduced oxidative stress, inflammation, and atherosclerosis. The amount of norepinephrine in the blood and the activity of the enzyme monoamine oxidase A (MAO-A) were both increased as a result of the persistent sympathetic hyperactivity impeded MAO-The development of atherogenic and proinflammatory particles was expanded in ECs because of ROS development and NF-B actuation brought about by the enactment of mitochondrial homeostasis. With the aid of NF-B and oxidative stress, it also inhibited PGC-1, a regulator of mitochondrial function. By disrupting the positive feedback regulation between mitochondrial dysfunction and inflammation brought on by RDN's inactivation of MAO-A, EC atheroprone phenotypic changes and atherosclerosis were prevented.

Keywords: Renal denervation; Endothelial dysfunction; Mitochondrial dysfunction; Inflammation; Atherosclerosis

Introduction

Atherosclerosis is the primary pathophysiology of coronary artery disease (CAD), the leading cause of death worldwide. Numerous comorbidities, including obesity, diabetes, hypertension, and aging, are risk factors for atherosclerosis. Atherosclerosis is remembered to rise up out of vascular endothelial brokenness, which is characterized by maladaptive changes in endothelial practical aggregates .Receptive oxygen species (ROS) are basically delivered by mitochondria, which are likewise a fundamental objective for harm welcomed on by ROS. The contribution of mitochondria, especially those tracked down in endothelial cells (ECs), in the improvement of atherosclerosis has not been entirely contemplated, regardless of the way that mitochondrial brokenness is a typical pathogenic system for some sicknesses. The disruption of mitochondrial redox homeostasis is the root cause of both innate immune signaling cascades that trigger inflammation and endothelial dysfunction. A reduction in inflammatory response, promotion of endothelium repair, and suppression of atherosclerosis are just a few of the potential advantages of strategies that reduce or neutralize the amount of ROS produced by mitochondria [1-5].

Endothelial dysfunction and activation of the sympathetic nervous system (SNS), according to recent research, frequently occur simultaneously. A recent study found that neurotransmitters may influence inflammatory leukocyte adhesion to ECs because arteries are innervated. This suggests that atherogenesis is also influenced by SNS. The sympathetic neurotransmitter norepinephrine is metabolized by the mitochondrial enzyme monoamine oxidase-A (MAO-A), which also produces hydrogen peroxide (H2O2) as a byproduct of this enzymatic reaction. However, there is an ambiguous connection between inflammation, oxidative stress, and sympathetic hyperactivity. It is interesting to note that MAO-A activation is linked to increased sympathetic drive and spillover of norepinephrine. Mitochondrial oxidative damage is responsible for the pathophysiology of vascular injury in atherosclerosis as a result of the excessive production of H2O2 in this phase. P53, which acts as a suppressor of peroxisome-proliferator-activated receptor-coactivator-1 (PGC-1), a crucial link between inflammatory, redox, and metabolic regulatory pathways, is one of the primary players in the MAO-A/H2O2 axis. The fact that endothelium contains more MAO-A suggests that an inflammatory response in ECs can be triggered by persistent MAO-A/H2O2 axis activation in the presence of chronic sympathetic hyperactivity [6-10].

Renal denervation (RDN) successfully reduces systemic sympathetic activity by severing the sympathetic nerves that travel through the renal artery's adventitia. It has been used as a novel treatment for resistant hypertension in therapeutic settings. In addition, our previous findings indicate that RDN improves endothelial function in the context of diabetes and has demonstrated potential therapeutic benefits on oxidative stress, inflammation, and insulin resistance-risk factors for endothelial dysfunction. Our objective in this study is to determine whether RDN can reduce atherosclerosis by improving endothelial function, specifically through pathways connected to the vicious cycle of MAO-A-induced mitochondrial ROS release and inflammation in ECs.

Subjective Heading

The First Affiliated Hospital of the University of Science and Technology of China's Institute Research Ethics Committee accepted the use of human serum samples in the experiments, which were carried out in compliance with the second principle of the Helsinki Declaration.

***Corresponding author:** Jihong Han, Key Laboratory of Metabolism and Regulation for Major Diseases of Anhui Higher Education Institutes, Hefei University of Technology, Hefei, 230009, China, E-mail: jihong08@nankai.edu.cn

Received: 01-Nov-2022, Manuscript No. asoa-22-84180; **Editor assigned:** 03-Nov-2022, PreQC No. asoa-22-84180 (PQ); **Reviewed:** 17-Nov-2022, QC No. asoa-22-84180; **Revised:** 19-Nov-2022, Manuscript No. asoa-22-84180 (R); **Published:** 24-Nov-2022, DOI: 10.4172/asoa.1000189

Citation: Han J (2022) Atherosclerosis by Targeting the Mitochondria-Inflammation Circle. Atheroscler Open Access 7: 189.

Copyright: © 2022 Han J. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

After obtaining informed consent, blood samples were taken from CAD patients and non-CAD controls (14 men and 6 women, ages 50 to 68). (12 males, 8 females, 51–65 years of age). The specific inclusion/exclusion standards and medical characteristics of non-CAD controls and CAD patients were described in the Supplementary Materials.

Eight-week-old male ApoE-deficient (ApoE-/-) mice were maintained at the Nankai University Animal Center in Tianjin, China, where they had free access to food and water. The RDN group (RDN) and the sham surgery group were randomly assigned to two groups of mice after one week of acclimation (Sham). While mice in the Sham group underwent abdominal surgery without RDN, mice in the RDN group got bilateral RDN surgically and chemically. Following surgery, all mice were given a pro-atherogenic high-fat diet (HFD) for 20 weeks, which comprises 21% fat and 0.5% cholesterol. After the mice were sedated and put to death in a CO2 chamber at the conclusion of the experiment, blood, kidney, renal artery, and aorta sample were collected. The Animal Welfare Board of Canada approved each study [11-13].

Endothelial Basal Medium 2 (EBM-2, Lonza, USA), which contains 2% foetal bovine serum (FBS), 50 g/mL penicillin/streptomycin, and growth factors, was used to cultivate human aortic ECs (HAECs, Lonza, USA). Using Lipofectamine RNAiMAX or Lipofectamine 2000, cells that were 70–80% confluent were transfected with either a PGC-1 CRISPR expression vector or an MAO-A siRNA from Santa Cruz, USA. Cells were switched to EBM-2 media containing FBS after a 24 hour transfection, and the appropriate therapy was administered. Purchased from ATCC (Manassas, USA), human monocytic cell line THP-1 cells were grown in RPMI 1640 media with 10% FBS and 50 g/mL penicillin/streptomycin.

Mice were positioned in a laying position on a platform after being given a general anaesthetic with isoflurane, and their abdomens were then opened. The surface of the renal arteries were painted with phenol solution (10% in ethanol) for 5 minutes using a tiny brush after the renal arteries and veins were identified and all visible nerves along vessels were cut. The other side received the same treatment. The same treatment was performed on the mice in the Sham group, with the exception that the nerves were not severed and the renal arteries were treated with 0.9% saline rather than phenol solution. The evaluation of renal artery morphology by hematoxylin and eosin (H&E) staining and norepinephrine content in kidney or serum at the conclusion of the experiment served to demonstrate the successful RDN [14, 15].

Discussion

Following collection, mouse whole aorta and 6-mm frozen cross sections of the aortic root were made, and they were utilised to identify en face lesions and sinus lesions by Oil red O staining. Based on the standards for experimental atherosclerosis investigations outlined in the American Heart Association statement all the pictures taken under a microscope were used to quantitatively analyse the lesion area (intima) and Oil red O positive region. The area of necrotic cores, thickness of the fibrosis cap, and collagen content of the aortic root were also assessed using cross sections. Masson trichrome (Solarbio, China) with H&E staining. Through the use of dihydroethidium (DHE, Sigma, USA) staining, ROS levels in plaques were ascertained. TUNEL labelling, performed by Vazyme Biotech in China, was used to identify apoptotic cells. The Leica DM5000B microscope (Wetzlar, Germany) was used to take each picture, and Image J was used for quantitative analysis.

Renal arteries were embedded in paraffin, fixed in 4% paraformaldehyde, and cut into 4-m slices before immunohistochemical labelling. Briefly, slices were treated first with biotinylated goat anti-rabbit IgG and then with anti-tyrosine hydroxylase antibody (Proteintech, 25859-1-AP, 1:200). The stain strength (level of tyrosine hydroxylase staining) was graded using a blinded system as 0, negative, 1, weak, 2, mild, 3, moderate, or 4, strong.

Immunofluorescent staining was performed on frozen aortic root cross sections using primary antibodies against CD68 (Santa Cruz, sc-17832, 1:200), SM22 (Proteintech, 10493-1-AP, 1:200), CD31 (Santa Cruz, sc-376764, 1:200), MCP-1 (Proteintech, 66272-1-Ig, 1:200), endothelin-1 (AbcamHAECs were fixed in 4% paraformaldehyde, permeabilized by 0.5% Triton, blocked with 5% BSA and incubated with primary antibody against NF-κB (Santa Cruz, sc-8008, 1:200), 8-OHdG (Santa Cruz, sc-66036, 1:200), or PGC-1α (Proteintech, 66369-1-Ig, 1:200), followed by incubation with FITC or rhodamine-conjugated secondary antibody.HAECs or mouse tissues' total proteins were extracted using a protease inhibitor-containing protein lysis solution (PMSF plus cocktail). In order to evaluate protein expression by Western blot as reported an equal number of total proteins from each sample were employed. After scanning each Western blot image, the band density was semi-quantitatively examined.

A reverse transcription kit (Invitrogen, USA) was used to create cDNA from total RNA isolated from mouse tissues. Quantitative real-time PCR (qPCR) was then conducted using a SYBR green PCR master mix and primers with the sequences specified of the Supplementary Materials. GAPDH mRNA in the matched sample was used to normalise the expression of mRNA for each gene.

Using the Mindray Biochemical Analyzer BS-190, the levels of mouse serum triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein AI (Apo-AI), and apo-B were measured (Mindray, Shenzhen, China). An enzymatic technique was used to assess the quantities of free fatty acids (FFA) in mouse serum (Biolabo, France). With the aid of the appropriate test kits, the presence of the oxidative stress markers malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, glutathione (GSH) content, and total antioxidant capacity (T-AOC) in mouse serum was determined (Solarbio, China).

ELISA kits were used to test the levels of norepinephrine (Abnova, China), MAO-A (Abbexa, UK), mouse serum cytokines (R&D, USA), and MAO-A levels/activity (Mbbiology, China) in serum samples from humans. Following transfection and treatment, conditioned media from HAECs was gathered for cytokine measurement using ELISA kits from Thermo Scientific (USA). Following sacrifice, mouse thoracic aortic tissues were gathered, fixed in 2.5% glutaraldehyde, and then postfixed in 1% osmium teroxide for 2 hours. In accordance with standard protocol, samples were dehydrated in gradient ethanol solutions. An electron microscope was used to view the generated ultrathin slices (Hitachi, Japan).

The dichlorofluorescin diacetate (DCFDA, Solarbio, China) technique was used to measure the intracellular ROS generation. After treatment, cells were placed on 35 mm confocal culture dishes and exposed to DCFDA for 30 min at 37 °C. Immediately after, the cells were immediately observed using a confocal microscope (ZEISS, LSM710, Germany) with excitation/emission wavelengths of 480/530 nm.

Using the MitoSox red fluorescent dye, mitochondrial superoxide

production was assessed in intact cells (Invitrogen, USA). Following treatment, cells were stained for 15 minutes at 37 °C with 1.5 mmol/L MitoSox red and 10 ng/mL Hoechst blue dye (Solarbio, China), followed by two washes with PBS. A confocal microscope was used to measure the MitoSox fluorescence in random fields (ZEISS, LSM710, Germany). Using ImageJ, all acquired fluorescence pictures were examined.

Cell toxicity was measured as a percentage of cell viability using the widely used CCK-8 assay (Solarbio, China). Norepinephrine was administered to HEACs for 48 hours after they had been plated in 96-well dishes with 1 104 cells per well. Each well received a treatment, then 10 L of CCK-8 solution was added, and the wells were allowed to incubate for two hours. The absorbance at 450 nm was measured in each well using a microplate reader. Cell viability was normalised to the control group. The ROS levels in the RDN group were shown to be lower than those in the Sham group using DHE staining .DNA oxidative damage caused by the MAO-A/H2O2 axis can be detected using the 8OHdG staining method. RDN equally decreased aortic endothelial 8OHdG levels

Cell toxicity was measured as a percentage of cell viability using the widely used CCK-8 assay (Solarbio, China). Norepinephrine was administered to HEACs for 48 hours after they had been plated in 96-well dishes with 1 104 cells per well. Each well received a treatment, then 10 L of CCK-8 solution was added, and the wells were allowed to incubate for two hours. The absorbance at 450 nm was measured in each well using a microplate reader. Cell viability was normalised to the control group. The ROS levels in the RDN group were shown to be lower than those in the Sham group using DHE staining .DNA oxidative damage caused by the MAO-A/H2O2 axis can be detected using the 8OHdG staining method. RDN equally decreased aortic endothelial 8OHdG levels, revealing its crucial function in mitochondrial biogenesis, as well as genomic copy number. RDN dramatically raised the protein or mRNA levels of Nrf2 and Tfam expression (Fig. 4H and I). RDN therefore greatly boosted the expression of antioxidant proteins (SOD2, CAT, and GPx4) in aortas .As a result, these findings suggest that RDN can reduce mitochondrial oxidative stress via controlling the p53/PGC-1 pathway.

Norepinephrine plays a major role in mediating sympathetic activity. It's interesting to note that norepinephrine circulation and persistently elevated sympathetic tone can be caused by cardiovascular risk factors. The effect of norepinephrine on the viability of HAECs was initially assessed by the CCK8 assay in order to further determine the relationship between RDN and endothelial function. Norepinephrine had no impact on cell survival at low doses, but it drastically decreased it at 100 mol/L (Fig. S3A), demonstrating its cytotoxicity to ECs. In fact, norepinephrine raised intracellular ROS levels in HAECs in a concentration- and time-dependent manner, as demonstrated by DCFDA labelling (Figs. S3B and S3C). Norepinephrine significantly altered the shape of the mitochondria in conjunction with an increase in ROS generation. The examination of the images using the Mitochondrial Network Analysis (MiNA) toolset and the MitoTracker Red labelling show that the mitochondrial footprint and

Norepinephrine induces MAO-A expression in HAECs in a concentration-dependent manner, despite the fact that norepinephrine is the substrate for MAO-A activity. It's interesting to note that norepinephrine-induced ROS generation and DNA oxidative damage were significantly reduced when MAO-A expression was specifically decreased by siRNA . Additionally, norepinephrine-impaired mitochondrial functions were considerably enhanced by MAO-A siRNA. The effects of norepinephrine on mitochondrial membrane potential and ROS levels were counteracted by MAO-A siRNA. Through NF-B pathway activation, mitochondrial ROS generation can start an inflammatory response. Norepinephrine decreased the expression of I-B while increasing the expression of phosphorylated NF-B in HAECs .Consequently, norepinephrine increased NF-B nuclear translocation (Fig. 6B; S3D). However, norepinephrine mitigated the effects of diminished IB and elevated p-NF-B or enhanced NF-B nuclear translocation.

In a vicious cycle during inflammation, PGC-1 and NF-B moderate one another, and oxidative stress is a key factor. PGC-1 expression vector transfection effectively reduced norepinephrine-reduced IB and boosted NF-B phosphorylation and nuclear translocation in HAECs (Fig. 6L; S4E). Norepinephrine enhanced the levels of MCP-1 and IL-6 in the HAEC conditioned media, while PGC-1 overexpression prevented the increases from occurring because they were associated with NF-B expression .The stimulation of PGC-1 expression also prevented the adherence of THP-1 cells to HAECs that was caused by norepinephrine (Fig. S4D). All of the aforementioned information points to the possibility that inhibiting NF-B can reduce the oxidative damage that norepinephrine-induced mitochondrial dysfunction causes.

Conclusion

The fact that this study mainly concentrated on how RDN altered EC phenotypes as atherosclerosis progressed is a drawback. However, the proliferation and migration of smooth muscle as well as macrophage phenotypes can be modified by sympathetic signalling activation .Sympathetic hyperactivity may support numerous elements of atherogenesis in later stages, including atheroma development, progression, and rupture, given its functions in a variety of pathologic diseases. Further research is needed to understand the precise functions of RDN and MAO-A activity in different cell types during advanced atherosclerosis.

In conclusion, we identified the mechanisms underlying endothelial dysfunctions corrected by RDN during atherogenesis, laying the groundwork for the use of RDN in hypertensive patients whose atherosclerosis complicates their condition. Additionally, the discovery that elevated norepinephrine spillover controls EC phenotypes via a mitochondrial-inflammation circuit controlled by MAO-A may offer potentially lucrative therapeutic options for the therapy of atherosclerosis.

Acknowledgement

I would like to thank my Professor for his support and encouragement.

Conflict of Interest

The authors declare that they are no conflict of interest.

References

1. Shafi S, Ansari HR, Bahitham W, Aouabdi S (2019) The Impact of Natural Antioxidants on the Regenerative Potential of Vascular Cells. Front Cardiovascu Med 6: 1-28.
2. Ala-Korpela M (2019) The culprit is the carrier, not the loads: cholesterol, triglycerides and Apo lipoprotein B in atherosclerosis and coronary heart disease. Int J Epidemiol 48: 1389-1392.
3. Esper RJ, Nordaby RA (2019) cardiovascular events, diabetes and guidelines: the virtue of simplicity. Cardiovasc Diabetol 18:42.

4. Hansson GK, Libby P, Tabas I (2015) Inflammation and plaque vulnerability. J Intern Med 278: 483-493.

5. Adamson S, Leitinger N (2011) phenotypic modulation of macrophages in response to plaque lipids. Curr Opin Lipidol 22: 335-342.

6. Fu P, Birukov KG (2009) Oxidized phospholipids in control of inflammation and endothelial barrier. Transl Res 153: 166-176.

7. Lehti S (2018) Extracellular lipid accumulates in human carotid arteries as distinct three-dimensional structures with pro inflammatory properties. Am J Pathol 188:525-538.

8. Westerterp M (2017) Cholesterol accumulation in dendritic cells links the inflammasome to acquired immunity. Cell Metab 25: 1294-1304.

9. Wityk RJ, Lehman D, Klag M, Coresh J, Ahn H, et al. (1996) Race and sex differences in the distribution of cerebral atherosclerosis. Stroke 27:1974-1980.

10. Kajinami K, Akao H, Polisecki E, Schaefer EJ (2005) Pharmacogenomics of statin responsiveness. Am J Cardiol 96: 65-70.

11. Kataoka Y, John J, Wolski K, Uno K, Puri R, et al. (2015) Atheroma progression in hyporesponders to statin therapy. Arterioscler Thromb Vasc Biol 35:990-995.

12. Zavodni AE, Wasserman BA, McClelland RL, Gomes AS, Folsom AR, et al. (2014) Carotid artery plaque morphology and composition in relation to incident cardiovascular events: the Multi-Ethnic Study of Atherosclerosis (MESA). Radiol 271: 381-389.

13. Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, et al. (2010) Coronary artery calcium score and risk classification for coronary heart disease prediction. JAMA 303: 1610-1616.

14. Arad Y, Goodman KJ, Roth M, Newstein D, Guerci AD (2005) Coronary calcification, coronary disease risk factors, C-reactive protein, and atherosclerotic cardiovascular disease events: the St. Francis Heart Study. J Am Coll Cardiol 46: 158-165.

15. Dichgans M, Pulit SL, Rosand J (2019) Stroke genetics: discovery, biology, and clinical applications. Lancet Neurol 18: 587-599.