Mitochondrial Oxidative Stress Mediates Macrophage Pro-inflammatory Metabolic Switch in Atherosclerotic Vascular Disease in Aging

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ABSTRACT

Aging elevates cardiovascular disease risk, including atherosclerosis. Macrophages play crucial role vascular aging by promoting inflammation and atherosclerosis progression. Age-related increase in NOX4 NADPH oxidase expression correlates with mitochondrial dysfunction, inflammation, and atherosclerosis severity. We hypothesized that NOX4-dependent mitochondrial oxidative stress induces macrophage metabolic dysfunction and an inflammatory phenotype in aging-associated atherosclerotic disease. Aortic and brachiocephalic artery lesion areas were comparable in 5-month-old (young) Apoe<sup>-/-</sup> and Apoe<sup>-/-</sup> mice, increased significantly in 16-month-old (aged) mice, but were significantly lower in Apoe<sup>-/-</sup> mice. Aged Apoe<sup>-/-</sup> mice, atherosclerotic lesions had reduced CD11b<sup>+</sup> area, lower expression of CCL2, IL-1β, and IL-6, and fewer classically activated pro-inflammatory macrophages (CD80<sup>+</sup>CD68<sup>+</sup>). Spectral flow cytometry and t-SNE analysis revealed a significantly lower proportion of activated inflammatory macrophages and macrophage-like cells in atherosclerotic lesions of aged Apoe<sup>-/-</sup> compared to Apoe<sup>-/-</sup> mice. Macrophages from aged Apoe<sup>-/-</sup> mice had altered metabolic function. In contrast, macrophages from Apoe<sup>-/-</sup>/Nox4<sup>-/-</sup> mice were less glycolytic, more aerobic, and had preserved basal and maximal respiration and mitochondrial ATP production. Nox4<sup>-/-</sup> macrophages had lower mitochondrial ROS and reduced IL-1β secretion, compared with Apoe<sup>-/-</sup> mice. In aged Apoe<sup>-/-</sup> mice, inhibition of NOX4 using GKT137831 significantly reduced macrophage ROS and improved mitochondrial function. This resulted in a decreased CD80<sup>+</sup>CD80<sup>-/-</sup> and increased CD163<sup>+</sup>CD206<sup>-/-</sup> macrophages and attenuated atherosclerosis.

Our results imply that NOX4-dependent mitochondrial oxidative stress in aging contributes to macrophage mitochondrial dysfunction, glycolytic metabolic switch, and pro-inflammatory phenotype, advancing atherosclerosis. Inhibition of NOX4 could alleviate vascular inflammation and atherosclerosis by improving mitochondrial function in macrophages.

RESULTS

Figure 1. Aging-associated atherosclerosis burden is attenuated in Nox4-deficient Apoe<sup>-/-</sup> mice. (A) Flow cytometry analysis and quantification of atherosclerotic lesion area in young (5-month-old) and aged (16-month-old) Apoe<sup>-/-</sup> and Apoe<sup>-/-</sup> mice fed Western diet for 3 months (mean±SEM, n=8). (B) Representative images of oil red-O-stained atheroma sections and quantification of atherosclerotic lesion area (mean±SEM, n=7). (C) Representative fluorescence microscopy images and fluorescence quantification in brachiocephalic artery sections stained with MitoSOX. Data are mean±SEM, n=7.

Figure 2. Increased NOX4 expression in aging is associated with vascular inflammation. (A) Representative fluorescence microscopy images and quantification of immunoreactive CD11b<sup>+</sup> expression (red) in brachiocephalic artery sections stained for ACTA2 (green) and DAPI (blue). Data are fluorescence integrated density of expression (ROI area x 10<sup>3</sup>) per lesion cell number (mean±SEM, n=6).

Figure 3. Nox4 deficiency induces pro-resolving phenotype in atherosclerotic lesion macrophages in aged Apoe<sup>-/-</sup> mice. (A) Oxygen consumption rate (OCR) measurements and quantification of atherosclerotic lesion single-cell respiration and mitochondrial ATP production. Nox4<sup>-/-</sup> macrophages had reduced mitochondrial OCR and ATP production.

Figure 4. Mitochondrial function and metabolic profiling of macrophages from young and aged Apoe<sup>-/-</sup> and Nox4<sup>-/-</sup> Apoe<sup>-/-</sup> mice. (A-C) Oxygen consumption rate (OCR) measurements and quantification of atherosclerotic lesion single-cell respiration and mitochondrial ATP production. Nox4<sup>-/-</sup> macrophages had reduced mitochondrial OCR and ATP production.

Figure 5. Inhibition of NOX4 improves mitochondrial function inducing pro-resolving phenotype in atherosclerotic lesion macrophages in aged Apoe<sup>-/-</sup> mice. (A) Quantification of MitoSOX fluorescence in control M0, M[IFNγ+LPS], and M[IL4] macrophages isolated from young and aged Apoe<sup>-/-</sup> and Nox4<sup>-/-</sup> Apoe<sup>-/-</sup> mice (mean±SEM, n=4). (B) Concentration of IL1β in conditioned media from M[IFNγ+LPS] macrophages (mean±SEM, n=4). (C) Quantification of MitoSOX fluorescence in cultured control M0, M[IFNγ+LPS] or M[IL4] macrophages pre-treated with vehicle or GKT137831 (mean±SEM, n=6). (D) Mitochondrial bioenergetic parameters were determined in control M0 (A), M[IFNγ+LPS] (B), and M[IL4] (C) cultured macrophages (mean±SEM, n=6). (E-G) Metabolic profiling showing basal respiration and glycolysis relations in control M0 (D), M[IFNγ+LPS] (E), and M[IL4] (F) cultured macrophage (mean±SEM, n=6).

Figure 6. Inhibition of NOX4 improves mitochondrial function inducing pro-resolving phenotype in atherosclerotic lesion macrophages in aged Apoe<sup>-/-</sup> mice. (A) Quantification of MitoSOX fluorescence in control M0, M[IFNγ+LPS], and M[IL4] macrophages isolated from young and aged Apoe<sup>-/-</sup> and Nox4<sup>-/-</sup> Apoe<sup>-/-</sup> mice (mean±SEM, n=4). (B) Concentration of IL1β in conditioned media from M[IFNγ+LPS] macrophages (mean±SEM, n=4). (C) Quantification of MitoSOX fluorescence in cultured control M0, M[IFNγ+LPS] or M[IL4] macrophages pre-treated with vehicle or GKT137831 (mean±SEM, n=6). (D) Mitochondrial bioenergetic parameters were determined in control M0 (A), M[IFNγ+LPS] (B), and M[IL4] (C) cultured macrophages (mean±SEM, n=6). (E-G) Metabolic profiling showing basal respiration and glycolysis relations in control M0 (D), M[IFNγ+LPS] (E), and M[IL4] (F) cultured macrophage (mean±SEM, n=6).

CONCLUSIONS

- Aging-associated increase in NOX4 expression/activity leads to mitochondrial dysfunction in macrophages, a metabolic shift towards glycolysis, and a proinflammatory phenotype.
- An inflammatory plaque microenvironment causes lesion expansion in aging.
- Reducing the expression/activity of NOX4 or improving mitochondrial function may help alleviate vascular inflammation and atherosclerosis.