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Research paper

Apolipoprotein A-1 mimetic peptide 4F promotes endothelial repairing and compromises reendothelialization impaired by oxidized HDL through SR-B1

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ABSTRACT

Disruption of endothelial monolayer integrity is the primary instigating factor for many cardiovascular diseases. High density lipoprotein (HDL) oxidized by heme enzyme myeloperoxidase (MPO) is dysfunctional in promoting endothelial repair. Apolipoprotein A-1 mimetic 4F with its pleiotropic benefits has been proven effective in many *in vivo* models. In this study we investigated whether 4F promotes endothelial repair and restores the impaired function of oxidized HDL (Cl/NO_2-HDL) in promoting re-endothelialization. We demonstrate that 4F and Cl/NO_2-HDL act on scavenger receptor type I (SR-B1) using human aorta endothelial cells (HAEC) and SR-B1 $^{(-/-)}$ mouse aortic endothelial cells. Wound healing, transwell migration, lamellipodia formation and single cell migration assay experiments show that 4F treatment is associated with a recovery of endothelial cell migration and associated with significantly increased endothelial nitric oxide synthase (eNOS) activity, Akt phosphorylation and SR-B1 expression. 4F increases NO generation and diminishes oxidative stress. *In vivo*, 4F can stimulate cell proliferation and re-endothelialization in the carotid artery after treatment with Cl/NO_2-HDL in a carotid artery electric injury model but fails to do so in SR-B1$^{(-/-)}$ mice. These findings demonstrate that 4F promotes endothelial cell migration and has a potential therapeutic benefit against early endothelial injury in cardiovascular diseases.

1. Introduction

High Density Lipoprotein (HDL) levels demonstrate an inverse relation with incidence of coronary artery disease [1]. In addition to its primary action of reverse cholesterol transport [2] that causes atherosclerosis plaque regression [3–6], HDL has pleiotropic traits like anti-inflammatory [7–11], anti-oxidative [12,13], and anti-apoptotic properties [13,14]. HDL plays an essential role in maintaining endothelial monolayer integrity preventing endothelial dysfunction and injury in response to shear stress from disturbed flow patterns. This disruption of the endothelial monolayer integrity plays a crucial role in the initiation and propagation of atherosclerosis [15] and can be alleviated by the proliferation of neighboring endothelial cells (EC) and endothelial progenitor cells (EPC) [16–18]. HDL promotes endothelial repair by upregulating endothelial nitric oxide (NO) synthase (eNOS) and endothelium-dependent vasodilation [7,19], stimulation of endothelial

List of abbreviations: ApoA-1, Apolipoprotein A-1; Cl/NO_2-HDL, oxidized HDL; Cl-HDL, chlorinated HDL; NO_2-HDL, nitrated HDL; ECM, endothelial cell medium; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; HAECs, human aorta endothelial cells; HDL, high density lipoprotein; HETEs, hydroxyeicosatetraenoic acids; HODEs, hydroxyoctadecadienoic acids; HRP, horseradish peroxidase; HUVECs, human umbilical vein endothelial cells; LysoPC, lysophosphatidylcholine MAECs, mouse aortic endothelial cells; MPO, myeloperoxidase; PON1, paraoxonase-1; PCNA, proliferating cell nuclear antigen; ROS, reactive oxygen species; SR-B1$^{+/+}$, SR-B1 wild-type; SR-B1$^{-/-}$, SR-B1 deficient

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cell proliferation and migration [20–22] and EPC-mediated endothelial repair [20,23,24]. Also, HDL mediates re-endothelialization by promoting differentiation, inhibition of apoptosis, and dhesion of circulating EPC [25,26].

Scavenger receptor-B type-I (SR-BI) on endothelial cells are directly involved in HDL signaling by promoting endothelial cell migration and re-endothelialization in addition to its traditional function of mediating cholesterol and phospholipid movement between its ligands and cells [18]. This process is nitric oxide independent activation of Rac GTPase through the receptor and dependent on activation of src kinase, phosphatidylinositol 3-kinase, and p44/42 mitogen-activated protein kinase. Human EPC also express surface HDL receptor ecto-F1-ATPase

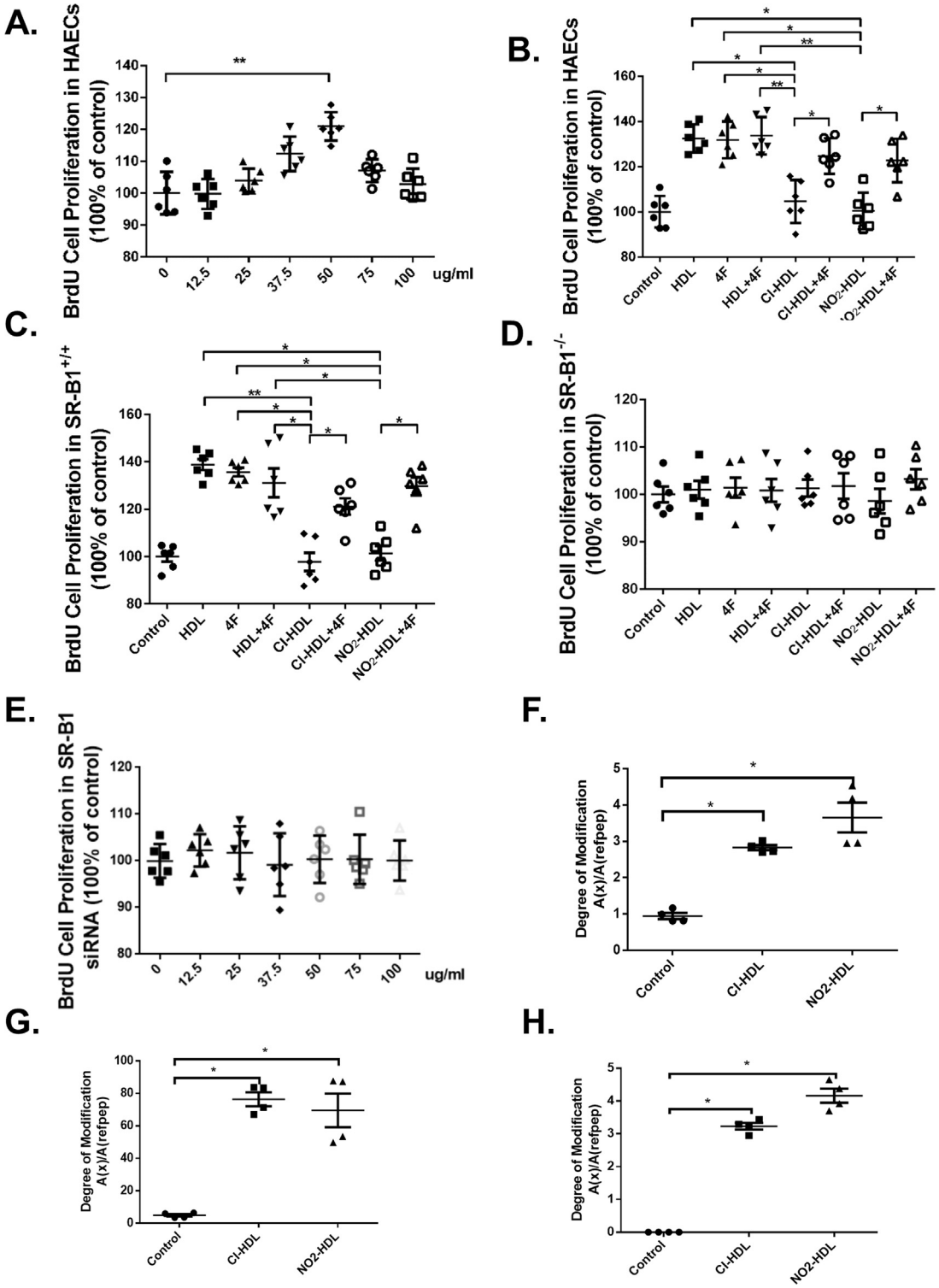

Fig. 1. Effects of 4F and SR-B1 on HAECs proliferation and Cl/NO_2-HDL function. HAECs were treated with 4F for 24 h at varying 4F concentrations in the range of 0–100 μg/mL. Relative cell proliferation was measured by BrdU cell proliferation assay (A). HAECs (B), aortic endothelial cells of SR-BI $^{(+/+)}$ mice (C), aortic endothelial cells of SR-BI $^{(-/-)}$ mice (D) and HAEC treated with SR-B1 siRNA (E). HDL oxidation (F) of tryptophan, (G) methionine, and (H) tyrosine were measured and expressed as oxidized apolipoprotein A-1 to native apolipoprotein A-1 ratios. The experiments had six independent biological replicates and every replicate have three technical replicates each. MAECs experiments were performed from three batches, and cells in each batch were isolated from three mice.