Redox Biology 19 (2018) 81–91

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Folic acid modulates *VPO1* DNA methylation levels and alleviates oxidative stress-induced apoptosis in vivo and in vitro

Shanshan Cui[a,1], Xin Lv[a,1], Wen Li[a], Zhenshu Li[a], Huan Liu[a], Yuxia Gao[b,*], Guowei Huang[a,*]

[a] Department of Nutrition and Food Science, School of Public Health, Tianjin Medical University, 22 Qixiangtai Road, Heping District, Tianjin 300070, China
[b] Department of Cardiology, General Hospital of Tianjin Medical University, Tianjin 300052, China

ARTICLE INFO

Keywords:
Folic acid
DNA methylation
Vascular peroxidase 1
Apoptosis
Oxidative stress
Atherosclerosis

ABSTRACT

Endothelial cell injury and apoptosis play a primary role in the pathogenesis of atherosclerosis. Moreover, accumulating evidence indicates that oxidative injury is an important risk factor for endothelial cell damage. In addition, low folate levels are considered a contributing factor to promotion of vascular disease because of the deregulation of DNA methylation. We aimed to investigate the effects of folic acid on injuries induced by oxidative stress that occur via an epigenetic gene silencing mechanism in ApoE knockout mice fed a high-fat diet and in human umbilical vein endothelial cells treated with oxidized low-density lipoprotein (ox-LDL). We assessed how folic acid influenced the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG, an oxidative DNA damage marker) and cellular apoptosis in in vivo and in vitro models. Furthermore, we analyzed DNA methyltransferase (DNMT) activity, vascular peroxidase 1 (VPO1) expression, and promoter methylation in human umbilical vein endothelial cells. Our data showed that folic acid reduced 8-OHdG levels and decreased apoptosis in the aortic tissue of ApoE$^{-/-}$ mice. Likewise, our in vitro experiments showed that folic acid protects against endothelial dysfunction induced by ox-LDL by reducing reactive oxygen species (ROS)-derived oxidative injuries, 8-OHdG content, and the apoptosis ratio. Importantly, this effect was indirectly caused by increased DNMT activity and altered DNA methylation at *VPO1* promoters, as well as changes in the abundance of VPO1 expression. Collectively, we conclude that folic acid supplementation may prevent oxidative stress-induced apoptosis and suppresses ROS levels through downregulating VPO1 as a consequence of changes in DNA methylation, which may contribute to beneficial effects on endothelial function.

1. Introduction

Cardiovascular diseases (CVDs) represent a major public health problem worldwide considering their high morbidity and disease burden. Among these, atherosclerotic cardiovascular disease remains a leading cause of death and disability [1,2]. It has been confirmed that endothelial cell injury and apoptosis play primary roles in the pathogenesis of atherosclerosis (AS) [3,4], and accumulating evidence indicates that oxidative injury represents an important risk factor for endothelial cell damage [5,6]. The subendothelial retention of low-density lipoprotein (LDL) and its oxidative modification, oxidized LDL (ox-LDL), activates signaling pathways resulting in an oxidative stress response, which eventually leads to AS [7,8]. Vascular peroxidase 1 (VPO1), a recently identified member of the peroxidase family in the cardiovascular system [9], contributes to oxidation and retention of LDL in vessel walls [10]. As a result, VPO1 may represent a novel mediator of AS. Therefore, uncovering the molecular mechanisms underlying oxidative stress-induced endothelial cell damage is of utmost clinical importance for the prevention and treatment of AS.

Recent findings suggest that the pathogenesis of AS involves dynamic changes in epigenetic markers and gene expression [11,12]. Furthermore, human studies have noted differentially methylated

Abbreviations: 5-mC, 5-methylcytosine; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AS, atherosclerosis; CAT, catalase; CpGs, cytosine-phosphate-guanines; CVD, cardiovascular disease; DNMT, DNA methyltransferase; FITC, fluorescein isothiocyanate; Gpx, glutathione peroxidase; HFD, high-fat diet; HUVECs, human umbilical vein endothelial cells; LDH, lactate dehydrogenase; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MDA, malondialdehyde; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; ox-LDL, oxidized low-density lipoprotein; PCR, polymerase chain reaction; ROS, reactive oxygen species; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; VPO1, vascular peroxidase 1; WT, wild-type

* Corresponding authors.
E-mail addresses: gaoyuxiatj@tmu.edu.cn (Y. Gao), huangguowei@tmu.edu.cn (G. Huang).
[1] The two authors contributed equally to the paper, so they are both first authors.

https://doi.org/10.1016/j.redox.2018.08.005
Received 13 June 2018; Received in revised form 2 August 2018; Accepted 7 August 2018
Available online 08 August 2018

cytosine-phosphate-guanines (CpGs) as an accompanying feature of AS [13–15]. DNA methylation is generally catalyzed by DNA methyltransferases (DNMTs), and methyl groups are added to the C5 position of cytosine residues; this is typically associated with transcriptional repression [16,17]. Importantly, epigenetic regulation is not static, as it can be altered by environmental stimuli such as nutrition and dietary supplements [18,19]. These findings imply a potential role for DNA methylation in AS. However, the epigenetic mechanisms remain to be further clarified.

Folic acid, an oxidized form of folate with high bioavailability, is the major component of one-carbon metabolism, including nucleotide metabolism, maintaining the cellular redox status and methylation metabolism [20]. Folate inadequacy has been linked to endothelial dysfunction and CVDs via epigenetic changes [21,22]. Epidemiological studies [23,24] have shown a decline in cardiovascular risk within countries with folic acid-fortified foods. The beneficial effect of folic acid is partially attributed to the epigenetic mechanisms in which folic acid acts as a methyl group donor. Moreover, a recent study has reported that folic acid is a micronutrient that can function as a novel redox regulator [25]. We also recently showed that folic acid alleviates atherogenesis by reducing plasma homocysteine levels and improving the antioxidant capacity in rats fed a high-fat diet (HFD) [26]; however, the mechanism underlying the relationship between folic acid and endothelial cell injury remains unclear.

Our previous study [27] has demonstrated that folic acid supplementation effectively prevents AS by regulating the normal homocysteine state, upregulating the S-adenosylmethionine (SAM): S-adenosylhomocysteine (SAH) ratio, and elevating DNMT activity. These results support that an increase in folic acid levels triggers epigenetic mechanisms to ameliorate AS. In the present study, we expanded our previous work to investigate the mechanism underlying the effects of folic acid on endothelial cell injury and apoptosis, employing ApoE knockout ($ApoE^{-/-}$) mice and human umbilical vein endothelial cells (HUVECs). Additionally, the primary goals of this study were to identify the epigenetic regulatory mechanisms underlying the folic acid-induced inhibition of VPO1 expression and to determine the redox signaling pathway by which folic acid attenuates endothelial cell injury.

2. Material and methods

2.1. Animals, diets, and experimental procedures

A total of 24 homozygous $ApoE^{-/-}$ mice from a C57BL/6 J background (male, 4 weeks old) and 8 age-matched male C57BL/6 J mice were purchased from Peking Huafukang Laboratory Animal Center (Beijing, China). $ApoE^{-/-}$ mice were randomly distributed into three groups (8 per group): (1) high-fat plus folic acid-deficient diet (HF + DEF), (2) HFD plus control diet (HF + CON), and (3) HFD plus daily intragastric gavage with 60 μg/kg body weight folic acid (HF + FA). In addition, 8 C57BL/6 J mice were used as wild-type (WT) controls. The folate-deficient diet (containing 0.2 mg folic acid/kg diet) and control diet (2.1 mg folic acid/kg diet) were purchased from TestDiet (St. Louis, MO, USA). All mice were fed a Western-type HFD [28] (21% fat, 1.25% cholesterol) and subjected to intragastric administration (folic acid or isometric 0.9% saline) for 20 weeks, when the mice reached the age of 24 weeks. The calculated nutritional composition of the HFD diet is listed in Supplementary Table 1. The mice were group housed in a temperature-controlled room (22.5 ± 0.5 °C) with a 12:12/h light/dark cycle and provided food and water ad libitum over the 20-week experimental period. The study was approved by the ethics committee of the Tianjin Medical University (TMUaMEC 2015009).

After 20 weeks, all mice were fasted overnight and then sacrificed via suffocation with CO_2. Blood was collected by cardiac puncture with a syringe and centrifuged for the preparation of plasma and serum. The plasma, serum, and aorta were immediately collected and stored at −80 °C until analysis.

2.2. HUVEC culture

HUVECs were obtained from GuangZhou Jennio Biotech Co., Ltd (Guangzhou, China) and were cultured in M199 medium (Yuanpei Biotechnology Co., Ltd, Shanghai, China) with 10% (v/v) fetal bovine serum (Gibco BRL, Grand Island, NY, USA), 100 IU/mL penicillin G, and 100 IU/mL streptomycin. The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and used at passages 3–5. HUVECs were exposed to the indicated concentrations of folic acid (0–1000 nmol/L) for 48 h and exposed to medium containing 120 μg/mL of ox-LDL or control vehicle for the first 24 h.

2.3. Immunofluorescence staining

Aortic arch samples fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned into consecutive 8-μm thick free-floating sections were de-waxed, hydrated, and then repaired using sodium citrate-EDTA. Sections were blocked with goat serum for 1 h at 37 °C and then reacted with mouse anti-8-hydroxy-2'-deoxyguanosine (8-OHdG) antibody (1:500; StressMarq Biosciences, Victoria, BC, Canada) at 4 °C overnight. The sections were washed with PBS and reacted with fluorescein isothiocyanate (FITC) -conjugated goat anti-mouse secondary antibodies (1:100) for 1 h at 25 °C. Antifade mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA) was used to dye the nuclei and for mounting. Images were obtained using a fluorescence microscope (Olympus, Tokyo, Japan). Positive cells were counted using Image Pro Plus 6.0 software.

2.4. TUNEL assay

Sections of the aortic arch were assayed with the Apoptosis Detection Kit (Roche, Basel, Switzerland). Sections were de-waxed, hydrated, blocked with Proteinase K solution for 20 min at 37 °C, and then reacted with TUNEL solution for 30 min at 37 °C in the dark. The sections were washed with PBS, reacted with Converter POD solution for 30 min at 37 °C in the dark, and visualized using a DBA Elite kit (Dingguo Changsheng Biotechnology Co., Ltd, Beijing, China). The images were obtained using microscopy (Olympus), and positive cells were counted using Image Pro Plus 6.0 software.

2.5. Measurement of plasma ox-LDL concentration

The plasma ox-LDL concentration was detected using the mouse ox-LDL ELISA Kit (CUSABIO TECHNOLOGY, Wuhan, China) according to the manufacturer's instructions. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibodies specific for ox-LDL were precoated onto a microplate. Then, the absorbance at 490 nm was recorded using a microplate reader (ELX800uv™; BioTek Instruments Inc, Winooski, VT, USA) within 5 min. Data were analyzed using the Curve Expert 1.3 software (CUSABIO TECHNOLOGY) to generate the standard curve.

2.6. DNMT activity assay

HUVEC nuclear extracts were isolated using the nuclear extraction kit (Merck, Darmstadt, Germany). DNMT activity was measured using a colorimetric DNMT activity/inhibition assay kit (Epigentek Group Inc., Farmingdale, NY, USA) according to the manufacturer's instructions. This sensitive ELISA-based method uses the ability of proteins containing methyl CpG-binding domains to bind methylated DNA with high affinity. The cellular protein content was determined using a BCA protein assay kit (BosterBio, Wuhan, China). Optical density (OD) was measured on a microplate reader at 450 nm, and DNMT activity [(OD)/