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Sulforaphane reduces advanced glycation end products (AGEs)-induced inflammation in endothelial cells and rat aorta

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Abstract *Background and Aims:* Advanced glycation end products (AGEs)-receptor RAGE interaction evokes oxidative stress and inflammatory reactions, thereby being involved in endothelial cell (EC) damage in diabetes. Sulforaphane is generated from glucoraphanin, a naturally occurring isothiocyanate found in widely consumed cruciferous vegetables, by myrosinase. Sulforaphane has been reported to protect against oxidative stress-mediated cell and tissue injury. However, effects of sulforaphane on AGEs-induced vascular damage remain unclear.
Methods and Results: In this study, we investigated whether and how sulforaphane could inhibit inflammation in AGEs-exposed human umbilical vein ECs (HUVECs) and AGEs-injected rat aorta. Sulforaphane treatment for 4 or 24 h dose-dependently inhibited the AGEs-induced increase in RAGE, monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecular-1 (VCAM-1) gene expression in HUVECs. AGEs significantly stimulated MCP-1 production by, and THP-1 cell adhesion to, HUVECs, both of which were prevented by 1.6 μM sulforaphane. Sulforaphane significantly suppressed oxidative stress generation and NADPH oxidase activation evoked by AGEs in HUVECs. Furthermore, aortic RAGE, ICAM-1 and VCAM-1 expression in AGEs-injected rats were increased, which were suppressed by simultaneous infusion of sulforaphane.
Conclusion: The present study demonstrated for the first time that sulforaphane could inhibit inflammation in AGEs-exposed HUVECs and AGEs-infused rat aorta partly by suppressing RAGE expression through its anti-oxidative properties. Inhibition of the AGEs-RAGE axis by sulforaphane might be a novel therapeutic target for vascular injury in diabetes.

List of abbreviations: AGEs, advanced glycation end products; RAGE, receptor for AGEs; Nrf2, nuclear factor erythroid-related factor 2; EC, endothelial cell; HUVECs, human umbilical vein ECs; BSA, bovine serum albumin; RT-PCR, reverse-transcription polymerase chain reactions; MCP-1, monocyte chemoattractant protein-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; RAGE-Ab, IgG polyclonal antibody directed against human RAGE; ELISA, enzyme-linked immunosorbent assay; HR, heart rate; BP, blood pressure; BG, blood glucose; AST, asparate aminotransferase; ALT, alanine aminotransferase; T-Chol, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; BUN, blood urea nitrogen; SEM, standard error; ROS, reactive oxygen species; DBP, diastolic BP; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

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Introduction

Sugars, including glucose, fructose and trioses can react non-enzymatically with the amino groups of proteins, lipids and nucleic acids to form reversible Schiff bases, and then Amadori products [1,2]. These early glycation products undergo further complex reactions such as rearrangement, dehydration and condensation to become irreversibly cross-linked, heterogeneous macroprotein derivatives called "advanced glycation end products (AGEs)" [1,2]. The formation and accumulation of AGEs in various tissues have been known to progress at a physiological normal aging process and at an accelerated rate under hyperglycemic and oxidative stress conditions [1,2]. Recent understandings of this process have revealed that AGEs and their receptor (RAGE) interaction evokes oxidative stress generation and inflammatory, thrombogenic and fibrotic reactions in a variety of cells, thereby playing a central role in vascular complications in diabetes [3–7].

Sulforaphane is generated from glucoraphanin, a naturally occurring isothiocyanate found in widely consumed cruciferous vegetables such as broccoli, kale, cabbage and brussels sprouts, by myrosinase [8]. Sulforaphane is an inducer of phase II anti-oxidant and detoxification enzymes with potential anti-cancer properties [8]. Recently, sulforaphane has been shown to protect against oxidative stress-mediated cell and tissue damage [8–11]. Indeed, sulforaphane has improved metabolic derangement, reduced albuminuria and inhibited glomerulosclerosis in type 1 diabetic rats by suppressing oxidative stress generation via activation of nuclear factor erythroid-related factor 2 (Nrf2) [9]. However, as far as we know, there is no paper to examine the effects of sulforaphane on AGEs-induced endothelial cell (EC) damage and vascular injury in animal models. Therefore, in this study, we investigated whether and how sulforaphane could inhibit inflammation in AGEs-exposed human umbilical vein ECs (HUVECs) and AGEs-injected rat aorta.

Methods

Materials

Sulforaphane and bovine serum albumin (BSA) (essentially fatty acid free and essentially globulin free, lyophilized powder) were purchased from Sigma (St. Louis, MO, USA). D-glyceraldehyde and normal rabbit IgG were purchased from Nakalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively.

Preparation of AGEs-BSA

AGEs-BSA was prepared as described previously [12]. In brief, BSA (25 mg/ml) was incubated under sterile conditions with 0.1 M glyceraldehyde in 0.2 M $NaPO_4$ buffer (pH 7.4) at 37 °C for 7 days. Then unincorporated sugars were removed by PD-10 column chromatography and dialysis against phosphate-buffered saline. Control non-glycated BSA was incubated in the same conditions except for the absence of reducing sugars as described previously [12].

Cells

HUVECs obtained from Lonza Group Ltd. (Basel, Switzerland) were cultured in endothelial basal medium supplemented with 2% fetal bovine serum, 0.4% bovine brain extracts, 10 ng/ml human epidermal growth factor and 1 μg/ml hydrocortisone according to the manufacturer's recommendation. AGEs or sulforaphane treatment was carried out in a medium lacking epidermal growth factor and hydrocortisone.

Real-time reverse transcription-polymerase chain reactions (RT-PCR)

HUVECs were treated with 100 μg/ml AGEs-BSA or non-glycated BSA in the presence or absence of the indicated concentrations of sulforaphane for 4 or 24 h. Then total RNA was extracted with RNAqueous-4PCR kit (Ambion Inc., Austin, TX, USA) according to the supplier's instructions. Quantitative real-time RT-PCR was performed using Assay-on-Demand and TaqMan 5 fluorogenic nuclease chemistry (Applied Biosystems, Foster city, CA, USA) according to the manufacturer's recommendation. IDs of primers for human RAGE, monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and 18S gene were Hs00542592_g1, Hs00234140_m1, Hs00164932_m1, Hs01003372_m1, and Hs99999901_s1, respectively.

Preparation of IgG polyclonal antibody directed against human RAGE (RAGE-Ab) for cell culture experiments

RAGE-Ab, which recognizes the amino acid residues 167–180 of human RAGE protein, was used for neutralizing assays and prepared as described previously [13].

MCP-1 production

HUVECs were treated with 100 μg/ml AGEs-BSA or non-glycated BSA in the presence or absence of the indicated concentrations of sulforaphane for 24 h. Then MCP-1 levels in the medium were measured with an enzyme-linked immunosorbent assay system (ELISA) (R&D Systems, Inc. Minneapolis, MN, USA).

Assay of THP-1 cell adhesion to HUVECs

Human THP-1 monocytic leukemia cells (American Type Culture Collection, Manassas, VA, USA) were maintained in RPMI 1640 medium supplemented with 1% GultaMAX (Life Technologies Corporation, Carlsbad, CA, USA) and 1% fetal bovine serum (NICHIREI BIOSCIENCES INC, Tokyo, Japan). THP-1 cells were labeled with 3 μM BCECF-AM (Dojindo, Kumamoto, Japan) at 37 °C for 30 min according to the supplier's recommendation as described previously [14].