SHORT COMMUNICATION

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High glucose-induced endothelial microparticles increase adhesion molecule expression on endothelial cells

Jamie G. Hijmans[1] · Tyler D. Bammert[1] · Kelly A. Stockelman[1] · Whitney R. Reiakvam[1] · Jared J. Greiner[1] · Christopher A. DeSouza[1]

Abstract

The experimental aim of this study was to determine, in vitro, the effects of glucose-induced EMPs on endothelial cell expression of E-selectin, intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 and platelet cell adhesion molecule-1 (PECAM-1). Human umbilical vein endothelial cells (HUVECs) were cultured (3rd passage) and plated in 6-well plates at a density of 5.0×10^5 cells/condition. HUVECs were incubated with media containing either 25 mM D-glucose (concentration representing a hyperglycemic state) or 5 mM D-glucose (normoglycemic condition) for 48 h to generate EMPs. EMP identification (CD144+) and concentration were determined by flow cytometry. HUVECs (3×10^6 cells/condition) were treated with either high glucose-derived EMPs (hgEMPs) or normal glucose-derived (ngEMPs) for 24 h and surface expression of E-selectin (CD62E-PE), ICAM-1 (CD54-FITC), VCAM-1 (CD106-APC) and PECAM-1 (CD31-BV) was assessed by flow cytometry and reported as mean fluorescent intensity (MFI). Hyperglycemic-derived EMPs induced significantly higher surface expression of E-selectin (2614 ± 132 vs. 2010 ± 204 MFI), ICAM-1 (2110 ± 81 vs. 1688 ± 152 MFI), VCAM-1 (3589 ± 431 vs. 2134 ± 386) and PECAM-1 (4237 ± 395 vs. 2525 ± 269 MFI) on endothelial cells than EMPs from normoglycemic conditions. Microparticle-induced cell adhesion molecule expression provides potential novel mechanistic insight regarding the accelerated risk of atherosclerotic vascular disease associated with hyperglycemia.

Keywords Glucose · Endothelial cells · Microparticles · Cell adhesion molecules

Introduction

Hyperglycemia is associated with an increased risk and prevalence of atherosclerotic cardiovascular disease (CVD) [1]. A major factor underlying the increased CVD burden associated with hyperglycemia is glucose-induced endothelial cell activation and dysfunction [2, 3]. A hallmark characteristic of endothelial cell activation, and in turn atherogenesis, is increased surface expression of cell adhesion molecules [4, 5]. Indeed, the adherence and subsequent transendothelial migration of circulating leukocytes and other inflammatory cells into the intima are predominantly mediated by the surface expression of adhesion molecules such as E-selectin, intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and platelet endothelial cell adhesion molecule (PECAM)-1 on the vascular endothelium. Morigi et al. [6] and others [7, 8] have demonstrated, in vitro, that high glucose concentrations upregulate the surface expression of these adhesion molecules, establishing glucose as a potent promoter of leukocyte adhesion and intimal infiltration [7]. High glucose also stimulates microparticle release from endothelial cells [9]. These endothelial-derived microparticles (EMPs) can confer deleterious autocrine effects on the endothelium [10–12]. For example, hyperglycemic-related EMPs have been shown to promote endothelial cell inflammation and apoptosis [11, 13, 14]. Moreover, EMPs have emerged as mediators and biomarkers of diabetes-related CVD risk and development [15, 16]. The effects of glucose-derived EMPs on endothelial adhesion molecule expression are not well defined. Accordingly, the aim of this study was to determine the effects of glucose-stimulated EMPs on endothelial cell surface expression of adhesion molecules.

✉ Christopher A. DeSouza
desouzac@colorado.edu

1   Integrative Vascular Biology Laboratory, Department of Integrative Physiology, University of Colorado, 354 UCB, 1725 Pleasant St, Boulder, CO 80309, USA

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Methods

Cell culture, EMP generation and enumeration

Human umbilical vein endothelial cells (HUVECs) were purchased from Life Technologies (ThermoFisher, Waltham, MA), and cultured in endothelial growth media (EBM-2 Bulletkit, Clonetics Lonza, Walkersville, MD), supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin (Life Technologies, Carlsbad, CA) under standard cell culture conditions (37 °C and 5% CO_2). HUVECs were cultured to the 3rd passage after reaching ~ 90% confluence and treated with media (RPMI 1640) containing 25 mM mannitol (to control for osmolality) and either 5 mM D-glucose (normal glucose) or 25 mM D-glucose (high glucose; representative of a diabetic glycemic state) for 48 h as previously described [14]. Thereafter, the supernatant from each condition was collected, centrifuged at $13,000 \times g$ at room temperature for 2 min to pellet and discard cellular debris, transferred to TruCount™ tubes (BD Biosciences, San Jose, CA) and incubated with CD144-PE (VE-Cadherin) for EMP determination. EMP size threshold was established using Megamix-Plus SSC calibrator beads (Biocytex, Marseille, France) [17]. EMP concentration (MP/µL) in the media from both conditions was determined by flow cytometry using the formula: [(number of events in region containing EMPs/number of events in absolute count bead region) × (total number of beads per test/total volume of sample)].

EMP-treated HUVECs

HUVECs were cultured as described above. Media containing EMPs harvested from either the normal glucose or high glucose conditions were centrifuged at $20,500 \times g$ for 30 min at 4 °C to pellet EMPs [11, 12, 14]. The pelleted EMPs were then re-suspended in EBM-2 at a concentration of 1.0×10^7 MPs/mL. HUVECs were then treated with an equal number of EMPs derived from either the normal glucose (ngEMPs) or high glucose (hgEMPs) conditions for 24 h.

Endothelial cell adhesion molecule surface expression

Cell surface expression of cell adhesion molecules was determined by flow cytometry. After EMP treatment, harvested HUVECs were incubated with CD62E-PE (E-selectin); CD54-FITC (ICAM-1); CD106-APC (VCAM-1); and CD31-BV (PECAM-1). Briefly, endothelial cells (2×10^5 per condition) were gated by their forward and side scatter profile. Biexponential scaling was used in all dot plots. Fluorescence minus one or isotype controls were used in all experiments. Mean fluorescence intensity (MFI) was standardized using Cytometer Setup and Tracking Beads (BD, Franklin Lakes, NJ, USA) and isotype negative control antibodies [18, 19].

Statistical analysis

Differences in glucose-treatment derived EMP number and adhesion molecule expression were determined by two-tailed, unpaired Student's t test. Data are reported as mean ± SEM for four independent HUVEC experiments. Statistical significance was set a priori at $P < 0.05$.

Results

The hyperglycemic condition elicited markedly higher (~ 260%; $P < 0.05$) EMP release compared with the normoglycemic condition (136 ± 38 vs. 37 ± 13 MP/µL) (Fig. 1). hgEMP exposure induced greater endothelial cell expression of each adhesion molecule. Surface expression of E-selectin (2614 ± 132 vs. 2010 ± 204 MFI), ICAM-1 (2110 ± 81 vs. 1688 ± 152 MFI), VCAM-1 (3590 ± 431 vs. 2134 ± 386 MFI) and PECAM-1 (4237 ± 395 vs. 2525 ± 269 MFI) was significantly higher in the hgEMP treated compared with ngEMP-treated cells (Fig. 2). Representative flow cytometry side scatter and histograms for EMP quantification and adhesion molecule expression, respectively, are shown in Fig. 3.
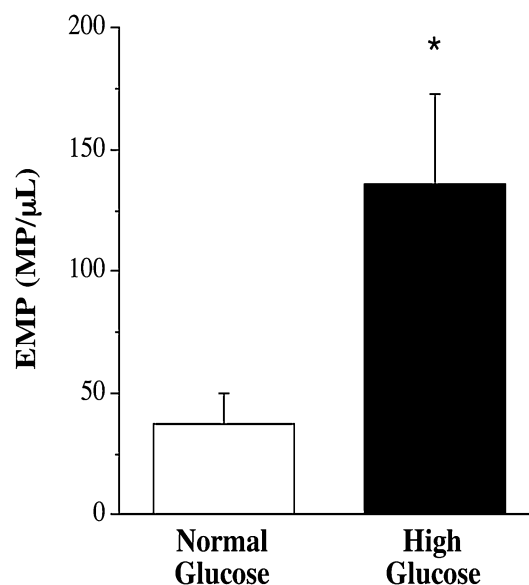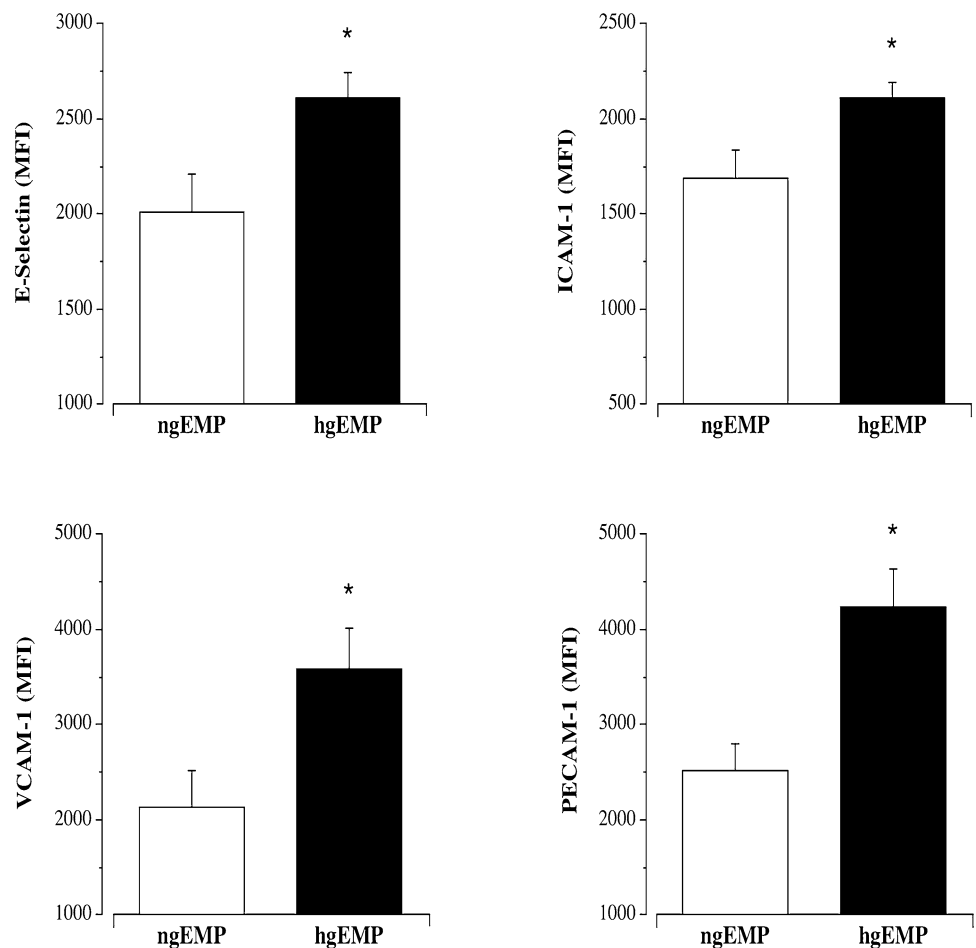

Fig. 1 EMP release in response to normal and high glucose concentrations. Values are mean ± SEM ($N = 4$). *$P < 0.05$

Fig. 2 Effect of EMPs generated from normal (ngEMPs) and high glucose (hgEMPs) concentrations on surface expression of E-selectin, ICAM-1, VCAM-1 and PECAM-1. Values are mean $\pm$ SEM ($N = 4$). *$P < 0.05$

Discussion

The novel finding of the present study is that high glucose-derived EMPs markedly increase endothelial cell surface expression of E-selectin, ICAM-1, VCAM-1 and PECAM-1. Elevated endothelial surface expression of these adhesion molecules is known to be an integral, precipitating event in the pathogenesis of atherosclerosis as well as an important contributor to plaque development and rupture [20, 21]. Several experimental [8, 22, 23] and clinical [24, 25] studies have demonstrated that hyperglycemia is associated with enhanced expression of cell adhesion molecules. While the direct effects of glucose on cell adhesion molecules are clear, to our knowledge, this is the first study to demonstrate that EMPs produced in response to high glucose stimulation also promote overexpression of adhesion molecules on the surface of endothelial cells.

Increased expression of cell adhesion molecules on the surface of endothelial cells has been shown to facilitate greater luminal leukocyte interaction, adhesion and migration into the subendothelial space, in turn, increasing the propensity for atherosclerosis [5]. Indeed, the tethering and rolling of leukocytes on the endothelial surface are controlled by selectins such as E-selectin, whereas leukocyte adhesion is mediated largely by immunoglobulins (e.g., ICAM-1 and VCAM-1) and transmigration through gap junctions is facilitated by PECAM-1 [26]. The pathologic consequence of this coordinated, complex interaction on the endothelial cell surface depends on the extent of adhesion molecule availability and presentation on the surface of endothelial cells [24, 25]. Surface expression of adhesion molecules is requisite for these interactions; as such, it is the evaluation of cell surface expression of adhesion molecules rather than general, non-location-specific cellular protein levels that is most important and physiologically relevant. The seminal and novel finding of the present study is that hgEMPs significantly increased the expression of E-selectin ($\sim 30\%$), ICAM-1 ($\sim 25\%$), VCAM-1 ($\sim 70\%$) and PECAM-1 ($\sim 70\%$) on the surface of endothelial cells. This finding is consistent with the notion that glucose-stimulated EMPs likely contribute to the increased risk of atherosclerotic vascular disease associated with hyperglycemic conditions [12]. The mechanisms underlying the hgEMP-induced increase in the surface expression of adhesion molecules are not clear. However, EMPs are important transport vectors for
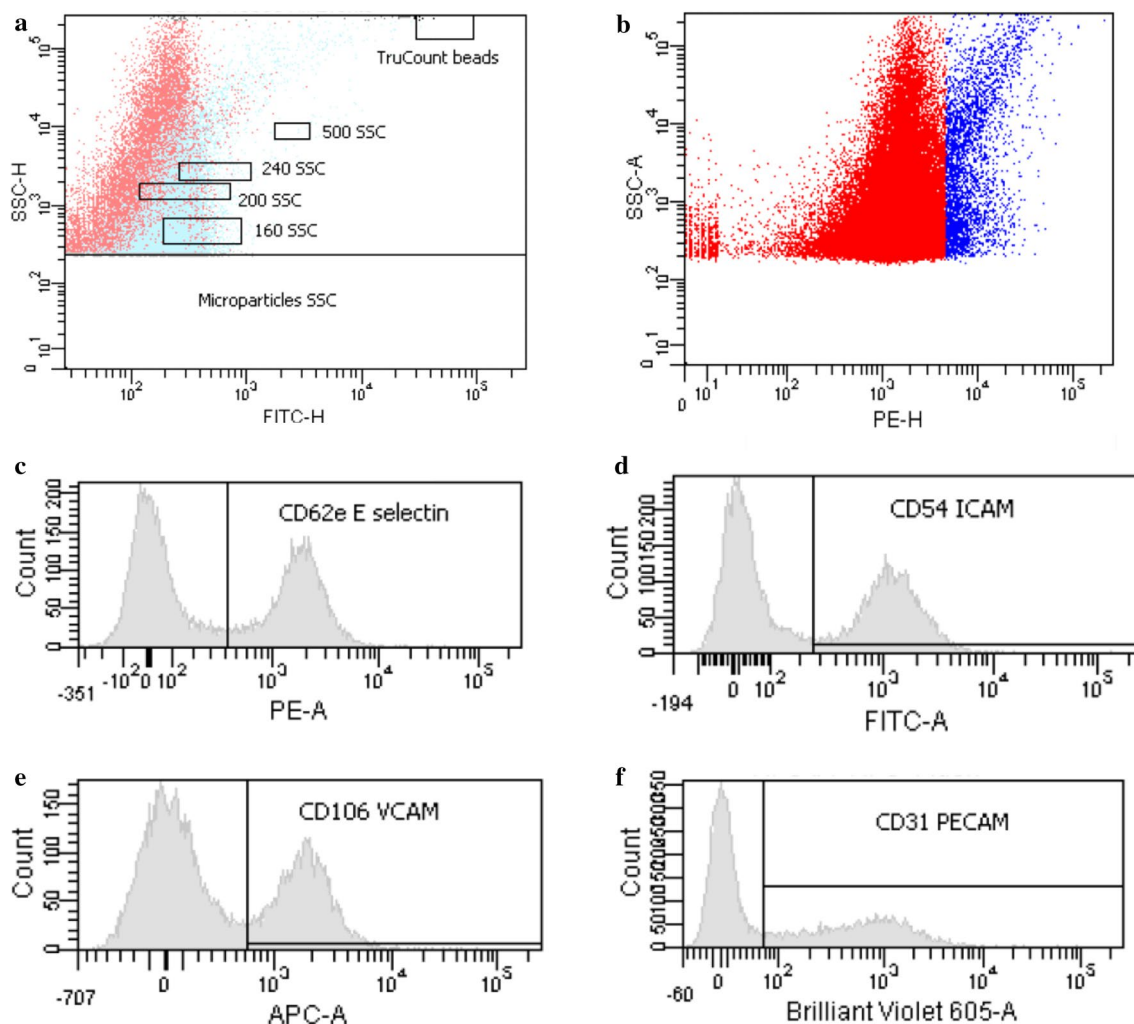
Fig. 3 Representative side scatter (SSC-H) by FITC height (FITC-H) (panel A) and PE height (PE-H) (panel B) dot plots showing the total number of microparticles and CD144+ microparticles. In addition, representative flow cytometric histograms showing cell surface expression of CD31-PECAM (panel B), CD62e-E-selectin (panel C), CD54-ICAM (panel D) and CD106-VCAM (panel. Mean fluorescence intensity (MFI) was established using Cytometer Setup and Tracking Beads (BD) and isotype negative controls

a myriad of proteins, cytokines and miRNAs that can disrupt target cell homeostasis. For example, Jansen et al. [11] demonstrated that EMPs-derived under high glucose conditions (similar to the present study) are pro-oxidative, with a cargo rich in NADPH oxidase and reactive oxygen species. This atherogenic microparticle phenotype was suggested to promote endothelial activation and adhesion molecule expression. Indeed, in their study, Jansen and colleagues [11] reported an increase in endothelial cell ICAM-1 and VCAM-1 protein levels in response to high glucose-derived EMPs. Our findings confirm increased expression of these adhesion molecules and significantly extend these findings demonstrating, for the first time, that increased expression involves greater endothelial cell surface density of not only ICAM-1 and VCAM-1 but also E-selectin and PECAM-1 [27]. The expression of cell adhesion molecules on the surface of endothelial cells is critical to atherogenesis. As such, our findings provide novel, more specific insight regarding the proatherogenic effects of EMPs that are produced under hyperglycemic conditions.

In summary, greater cell adhesion molecule expression on the surface of the endothelium heightens atherogenic potential and thrombotic risk [5]. Importantly, herein we demonstrate that EMPs generated from high glucose exposure induce an increase in cell adhesion molecules on the surface of endothelial cells. Our results provide additional, specific support for the notion that hyperglycemic-related EMPs contribute to the pathogenesis of vascular disease associated with diabetes. EMPs may represent an important target for therapeutic intervention aimed at reducing the risk of vascular disease in diabetic individuals.

Compliance with ethical standards

Ethical standards This study does not involve either human or animal subjects.

Conflict of interest Each author affirms no conflicts of interest in the conduct or reporting of this study.

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