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Modulation by high glucose of adhesion molecule expression in cultured endothelial cells

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Summary We evaluated the influence of high ambient glucose on cellular expression of adhesion molecules, known to mediate endothelial interaction of leucocytes and monocytes. Paired cultures of individual isolates of human umbilical vein endothelial cells (HUVECs) were studied by fluorescence activated cell sorter analysis after exposure to 30 vs 5 mmol/l glucose. Incubation of HUVECs for 24 h in 30 mmol/l glucose increased ICAM-1 (intercellular adhesion molecule-1; 116.4 ± 16.9 % of control, $p \leq 0.05$), but not PECAM (platelet endothelial cell adhesion molecule) expression, compared to cultures kept in 5 mmol/l glucose. Long-term exposure (13 ± 1 days) of HUVECs to 30 mmol/l glucose increased expression of ICAM-1 to 122.5 ± 32.2 % ($p < 0.002$) and reduced that of PECAM to 86.9 ± 21.3 % vs the respective control culture in 5 mmol/l glucose ($p < 0.02$). Stimulation of confluent HUVECs, kept in 30 vs 5 mmol/l glucose for 13 ± 1 days, with 20 U/ml interleukin-1 for 24 h (ICAM-1) and 4 h (endothelial leukocyte adhesion molecule 1) resulted in reduced ICAM-1 (84.8 ± 27.0 %, $p < 0.05$) and endothelial leukocyte adhesion molecule-1 (87.6 ± 22.4 %, $p < 0.05$) expression vs control cells, while that of PECAM (t: 24 h) and vascular cell adhesion molecule-1 (t: 16 h) remained unchanged. In conclusion, it appears that differences in expression of adhesion molecules on HUVECs in response to high glucose reflects endothelial glucose toxicity, which may also induce endothelial dysfunction in diabetes. [Diabetologia (1995) 38: 1367–1370]

Key words High glucose, adhesion molecules, endothelial cells, interleukin-1, diabetes mellitus.

Both insulin-dependent and non-insulin-dependent diabetes mellitus are associated with an increased risk for atherosclerosis. Endothelial dysfunction, which precedes the development of atherosclerotic lesions in diabetic patients, includes accelerated disappearance of capillary endothelium, weakening of intercellular junctions, altered protein synthesis and the appearance of specific adhesive glycoproteins on endothelial cells [1], promoting local attachment of monocytes and leukocytes as well as their transendothelial migration. The mechanisms leading to elevated plasma levels of shed soluble adhesion molecules in diabetes are unknown and may include their increased synthesis due to disease-specific factors, decreased clearance or just reflect the presence of inflammatory processes. As blood glucose levels, glycated haemoglobin and late diabetes-associated vascular complications are closely correlated [2], this study was designed to evaluate the effect of high ambient glucose concentrations on basal and interleukin-1 (IL-1) stimulated expression of the endothelial adhesion molecules, intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule

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Abbreviations: HUVECs, Human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; PECAM, platelet endothelial cell adhesion molecule; ELAM-1, endothelial leukocyte adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; IL-1, interleukin-1; FACS, fluorescence activated cell sorter; FCS, fetal calf serum; PBS, phosphate buffered saline.

Table 1. Identification of adhesion molecules on cultured HUVECs by FACS analysis and median fluorescence intensity following exposure to 5 and 30 mmol/l glucose with and without added IL-1

Adhesion molecule	*n*	Fluorescence intensity Glucose			*p*	Interleukin-1 Duration of exposure
		5 mmol/l	30 mmol/l	Duration of exposure		
ICAM-1	7	20.3 ± 6.6	23.6 ± 7.5	24 h	≤ 0.05	–
PECAM	7	29.5 ± 5.9	30.2 ± 7.1	24 h	NS	–
ICAM-1	25	25.8 ± 6.6	31.5 ± 10.9	13 ± 1 days	< 0.002	–
PECAM	20	34.4 ± 8.1	28.8 ± 5.8	13 ± 1 days	< 0.02	–
ICAM-1	19	17.3 ± 3.3	14.4 ± 5.0	13 ± 1 days	< 0.05	24 h
PECAM	11	42.8 ± 8.8	43.2 ± 10.1	13 ± 1 days	NS	24 h
ELAM-1	13	24.2 ± 9.1	20.4 ± 7.3	13 ± 1 days	< 0.05	4 h
VCAM-1	14	20.3 ± 5.3	22.5 ± 8.3	13 ± 1 days	NS	16 h

Values are mean ± SD

(ELAM-1), vascular cell adhesion molecule-1 (VCAM-1), and platelet endothelial cell adhesion molecule-1 (PECAM).

Materials and methods

Cell culture. Human umbilical vein endothelial cells (HUVECs) were isolated by collagenase treatment of umbilical veins and cultured on gelatin-coated culture dishes in Medium 199 (Sigma, St. Louis, Mo., USA), containing 20 % fetal calf serum (FCS, Gibco, Grand Island, N. Y. USA), 60 mg/l endothelial cell growth supplement (Technoclone, Vienna, Austria), penicillin-streptomycin (100 mg/l), fungizone (0.5 mg/l) and heparin (5×10^6 mU/l) as described previously [3]. Supplemented medium 199/20 % FCS was used for all experiments including short-term and long-term incubation with high vs normal glucose concentration, with the only exception being a change to supplemented medium 199/5 % FCS 16 h before and during IL-1 stimulation. The cultures were maintained at 37 °C and 5 % CO_2 and subcultures were performed using trypsin-EDTA. For all experiments endothelial cells were used as paired cultures of individual isolates exclusively in the first subculture. Endothelial cells were identified by their typical phase contrast "cobblestone" morphology and by immunofluorescence to von Willebrand factor antigen.

Short-term incubation with high glucose. After exposure of confluent HUVECs to high (30 mmol/l) or low (5 mmol/l) glucose or 30 mmol/l mannose (as osmotic control) for 24 h, adhesion molecule surface expression was determined by fluorescence activated cell sorter (FACS) analysis.

High glucose long-term culture. For all cultures assigned for long-term glucose experiments, control cells (5 mmol/l glucose) were plated at 1×10^5 cells per 60-mm dish, whereas companion cells (from the same identical individual isolate) in high glucose were plated with 30 % higher density, implemented to study experimental and control cells in the same assay in the same final density, as replication of HUVECs is delayed by high ambient glucose. At morphological confluency cells were analysed for cell numbers and adhesion molecule surface expression.

Incubation of HUVECs with IL-1. Confluent HUVECs, grown in 5 or 30 mmol/l glucose, respectively, were washed twice with phosphate buffered saline (PBS) and supplemented with medium 199/5 % FCS for 16 h before addition of IL-1. After a further washing step with PBS, HUVECs were incubated in medium 199/5 % FCS containing 20 U/ml IL-1α (Boehringer Mannheim, Mannheim, Germany) for 4 h (ELAM-1), 16 h (VCAM-1) or 24 h (ICAM-1 and PECAM), previously found to give maximum stimulation of the respective adhesion molecules (ELAM-1, VCAM-1, ICAM-1) in HUVECs [4].

Expression of adhesion molecules on endothelial cell surface. This was analysed by fluorescence-activated flow cytometry on a Becton Dickinson FACS-STAR (Becton Dickinson, Heidelberg, Germany). After washing with PBS, cells were detached using ice-cold PBS, thoroughly resuspended and exposed to 50 µl of either ICAM-1-(Clone RR 1/1 Bender, Vienna, Austria). PECAM-, ELAM-1- or VCAM-1-(all three from Immunotech, Marseille, France)-monoclonal antibody for 30 min each in melting ice. After addition of 1-ml FACS washing solution (5 % FCS, 0.1 mmol/l NaN_3 in PBS) cells were washed twice with the above solution and exposed to 50 µl of the fluorescein-labelled second antibody (Dako, Glostrup, Denmark) for 30 min on melting ice. Following a further washing step with FACS washing solution the cells were analysed. Since both primary and secondary antibody were used at saturating concentration, endothelial cell-membrane antigen expression could be quantitated as a measure of median fluorescence intensity. Specific fluorescence intensity was corrected for unspecific fluorescence by parallel incubation of each individual sample with second antibody without specific first antibody.

Statistcal analysis

Data are given as mean values ± SD and non-parametric statistical analysis was by Wilcoxon signed-rank test for median fluorescence intensities.

Results

Identification of adhesion molecules on cultured HUVECs was performed by fluorescence activated flow cytometry following exposure to 5 and 30 mmol/l glucose with and without additon of IL-1 (Table 1).

High glucose short-term culture. Short-term 24-h incubation of confluent HUVECs ($n = 7$) with high glucose (30 mmol/l) resulted in increased ICAM-1 surface expression (116.4 ± 16.9 % of control cells;

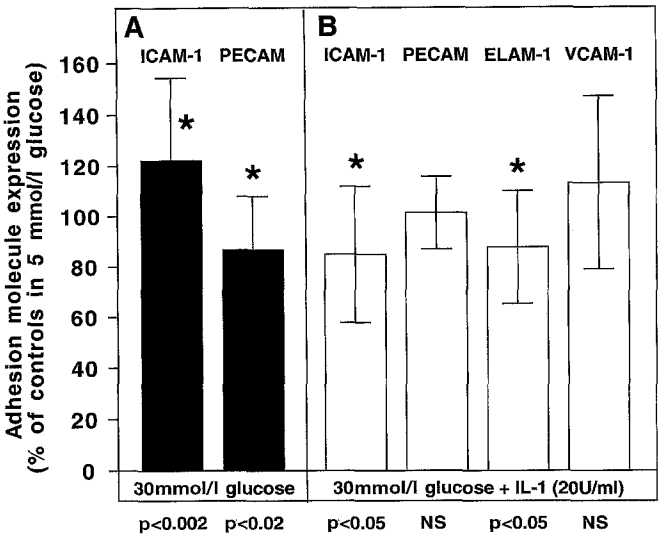

Fig. 1 A, B. Modulatory effect of long-term (13 ± 1 days) culture of HUVECs in 30 vs 5 mmol/l glucose (control) on expression **A**) of ICAM-1 and PECAM, and **B**) of IL-1 (20 U/ml) stimulated endothelial adhesion molecules (ICAM-1, PECAM, ELAM-1 and VCAM-1)

$p \leq 0.05$), whereas that of PECAM was not changed (103.8 ± 26.9 % of control; p = NS). Mannose (30 mmol/l), used as osmotic control, did not significantly affect ICAM-1 expression (106.0 ± 11.9 % of control; p = NS).

Long-term high glucose culture (Fig. 1 A). Cell counts determined in confluent HUVECs after 13 ± 1 days by trypan blue staining were: for 5 mmol/l glucose: $1.0 \pm 0.3 \times 10^6$; for 30 mmol/l glucose: $1.13 \pm 0.3 \times 10^6$; for 30 mmol/l mannose: $1.06 \pm 0.3 \times 10^6$ (p = NS) per 60-mm dish. Exposure of HUVECs to 30 mmol/l glucose for 13 ± 1 days increased their expression of ICAM-1 (n = 25; 122.5 ± 32.2 % of control; $p < 0.002$) and reduced that of PECAM by 13 % (n = 20; 86.9 ± 21.3 % of control; $p < 0.02$). Cultures kept in 30 mmol/l mannose did not exhibit changes vs controls in expression of ICAM-1 (n = 21; 102.8 ± 21.6 %; p = NS) and PECAM (n = 21; 93.3 ± 18.5 %; p = NS).

Effect of IL-1 (Fig. 1 B). Stimulated expression of adhesion molecules (ICAM-1, ELAM-1 and VCAM-1) by HUVECs following addition of IL-1 (20 U/ml) after prior exposure to 30 mmol/l glucose for 13 ± 1 days was reduced for ICAM-1 (n = 19; 84.8 ± 27.0 %; $p < 0.05$) and ELAM-1 (n = 13; 87.6 ± 22.4 %; $p < 0.05$) vs control cells kept in 5 mmol/l glucose. VCAM-1 (n = 14; 113.1 ± 34.17 %; p = NS) and PECAM (n = 11; 101.3 ± 14.4 %; p = NS) stimulation by IL-1 remained however unaffected by long-term culture in 30 vs 5 mmol/l glucose. No change in expression of adhesion molecules was induced by high mannose concentration (30 mmol/l).

Discussion

Elevated plasma levels reflecting release and clearance of circulating adhesion molecules have been linked to the development of atherosclerosis in diabetes [5]. Associated endothelial binding of monocytes and lymphocytes is by ELAM-1, ICAM-1 and VCAM-1, while neutrophils also bind to ELAM-1 and ICAM-1 [6]. In addition to endothelia, PECAM-1 is expressed on platelets, monocytes, polymorphonuclear cells, neutrophils and T-cell subsets [7].

In this context it is of note that exposure of HUVECs to 30 vs 5 mmol/l glucose – as shown in this study – increased endothelial expression of ICAM-1 both during short-term (24 h: + 16 %) and long-term (13 ± 1 days: + 22 %) incubation. In parallel, expression of PECAM was unaffected by 24-h exposure, but reduced (–13 %) by long-term exposure to 30 mmol/l glucose (Fig. 1 A). That finding is remarkable, as PECAM is concentrated in cell-to-cell contact areas and known to contribute to intercellular adhesion mediated by molecules of the IgG superfamily [8]. In IL-1β activated endothelium, its greatest adhesive strength for leukocytes is exerted in the junctional region [9], which implies that any down-regulation of PECAM expression by high glucose could weaken cellular junctions and trigger extravasation of polymorphonuclear cells out of the circulation in vivo.

Increased monocyte binding by human aortic endothelial cells seen in response to 25 mmol/l glucose, without any induction of major endothelial cell adhesion molecules [10], correlates with the absence of expression of ELAM-1 and VCAM-1 on HUVECs in response to 30 mmol/l glucose (data not shown). The failure of human aortic endothelial cells to also express ICAM-1 may depend on differences in applied methodology as in situ ELISA [10] vs flow cytometry, in the site of origin of endothelial cell type being either aortic or umbilical (HUVECs) and in regional endothelial dynamic response. In addition, use of HUVECs during the first passage instead of passages 4 to 9 in human aortic endothelial cells and adjustment of plating density to correct for high glucose-induced replicative delay may have provided for more marked expression of ICAM-1 on endothelial cells in response to 30 mmol/l glucose in our study. Since glucose was reported to activate protein kinase C and some lipoxygenase enzymes, lipid oxidation has been suggested by Kim et al. [10] to be co-responsible for associated monocyte binding to the endothelium.

Prolonged exposure to high ambient glucose (30 mmol/l) also interferes with cytokine-mediated adhesion molecule expression by down-regulation of IL-1 stimulated ICAM-1 (– 15 %) and ELAM-1 (– 13 %) expression on HUVECs (Fig. 1 B). The concomitant failure of 30 mmol/l glucose to also affect cytokine-stimulated expression of PECAM and

VCAM-1 again implies that high glucose selectively interferes with endothelial function.

In summary, we conclude that the modulation by high glucose of the expression of adhesion molecules on HUVECs describes a further component of glucose toxicity [1], which may be of importance for the development of endothelial damage in the diabetic state in vivo.

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