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Combined effects of shear stress and glucose on the morphology, actin filaments, and VE-cadherin of endothelial cells in vitro

Erna Sulistyowati [a,*], Nur Permatasari [b], M. Aris Widodo [b]

[a] Department of Pathology, Faculty of Medicine, Malang Islamic University, Malang, East Java, Indonesia
[b] Department of Pharmacology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

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ABSTRACT

Objective: The purpose of the present study was to analyze the effects of glucose and shear stress on the morphology and density of endothelial cells, actin filamen, and the expression of VE-cadherin.
Methods: After confluency of endothelial cells (3–4 days), 22 mM of D-glucose was administered for 7 days. Endothelial cells were exposed to shear stress of 10 dyne/cm^2 for varied durations of 5, 8, 12, and 15 min. Morphology of ECs was observed using an inverted microscope before and after shear stress exposure. VE-cadherin and actin filament were analyzed immunohistochemically.
Results: Exposure to high glucose induces more shrinkage and the cell density decreased at 15 min. High glucose reduced actin filaments and the more globular ones, especially around the nuclei. There was a decline in VE-cadherin scores with significant differences between treatments with 5 mM and 22 mM of glucose.
Conclusion: Combination exposure of shear stress and high glucose changes morphology, reduces actin filament and VE-cadherin, of endothelial cells.

1. Introduction

Until recently, atherosclerosis remains a problem in many parts of the world. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) have opposite roles in the pathomechanisms of atherosclerosis [1,2]. In most cases, atherosclerosis occurs in the bifurcation areas, bends, or stenosis of the artery. At these sites, blood flows are impaired in which the vortical flows and zones are separated. These events predispose to atherosclerosis. In addition to impaired blood flows, the slowing down of the blood flow may occur. This slowing down flow causes interaction between the low-density lipoprotein and blood vessel walls and then there is penetration into the vascular walls [3].

Vascular endothelial cells (ECs) are constantly exposed to the shear stress of blood flows. Endothelial cells are highly responsive to shear stress characterized by conversion of mechanic stimulus into biologically relevant signals (mechanostransduction). Early stages of this response include cell-anchoring integrins and activation of membrane receptors, such as vascular endothelial growth factor receptor-2 (VEGFR-2) and G-protein coupled receptors [4–7]. Subsequent to the initial activation of the molecules, the signals are transmitted into cells through the activation of various signal transduction pathways, such as mitogen-activated protein kinase (MAPK) pathway, protein kinase B (PKB/Akt) pathway, and endothelial nitric oxide synthase (eNOS) signals. The processes lead to functional changes of apoptosis and cell proliferation, inflammatory sensitivity, and cytoskeletal remodeling [8–10].

A variety of vascular complications such as micro and macroangiopathies often are found in individuals with type 1 diabetes mellitus with impaired blood sugar control. Incidence of vascular complications increases morbidity and mortality of patients [11]. Hyperglycemia represents one of the complication-accelerating factors, interacting with various factors of blood flow such as shear stress. To the best of our knowledge, no study has evaluated the combined effects of hyperglycemia and shear stress on ECs so far. The purpose of the present study was to analyze the effects of hyperglycemia and shear stress on the morphology and density of ECs. In addition, it will evaluate the effects of these factors on the expression of VE-cadherin.

2. Material and methods

2.1. Endothelial cells culture

This study was of experimental in vitro. Umbilical veins that have been cleaned with phosphate-buffered saline A (PBS-A) solutions were isolated enzymatically by the use of type II collagenase dissolved in a serum free medium consisting of M199 (Gibco), 100 μ/ml of penicillin and 100 μ/ml of streptomycin (Sigma), 21 mM/ml solution of sodium bicarbonate-phenol red, 2 mM glutamine. Cells were incubated for 7 min and rinsed with 10 ml of PBS-A. Solutions containing ECs were

* Corresponding author.
E-mail address: dr_erna@unisma.ac.id (E. Sulistyowati).

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centrifuged at 1000 rpm for 8 min in order to obtain pellets of ECs. ECs were grown on glass objects in 6-well culture plates. Upon formation of monolayers and confluence of endothelial cells (3–4 days), 22 mM of D-glucose was administered for 7 days. Media were replaced every 2 days.

2.2. Shear stress

Cone and plate were used for treatment with shear stress, as shown in Fig. 1. ECs were exposed to shear stress of 10 dyne/cm^2 for varied durations of 5, 8, 12, and 15 min. The variation of shear-stress duration was made between 5 and 15 min to determine their effects on changes in the morphology, density, the structure of VE-cadherin, and actin filaments of endothelial cells.

2.3. Endothelial morphology and density

Morphology of ECs was observed using an inverted microscope before and after shear stress. Cell density was counted manually under an inverted microscope on day 7 of treatment with 22 mM of glucose.

2.4. Immunohistochemistry

ECs grown on cover slips were washed 3 times with sterile PBS of pH 7.4 and fixed with methanol for 5–8 min. Then, each well was washed and incubated with sterile PBS of 2 ml for 5 min. Subsequently, it was washed 3 times with sterile PBS. Incubate with 1 ml of H_2O_2 3% for 10 min. Wash each well 3 times with 2 ml of sterile PBS for 5 min. Subsequently, each well was incubated with 1 ml of 5% FBS for 1 h. Incubate each well overnight with 1 ml of the primary antibody to VE-cadherin/actin filament mouse monoclonal IgG1 1:100. Wash each well 3 times with 2 ml of sterile PBS for 5 min. Incubate each well with 1 ml of Conjugated Gout Human Anti Mouse IgG adsorbed B Biotin of 1:200 for 1 h. Wash each well 3 times with 2 ml of sterile PBS for 5 min. Incubate each well with 1 ml of 1:500 SA-HRP for 40 min. Wash each well 3 times with 2 ml of sterile PBS for 5 min. Incubate each well with 1 ml of DAB (diamino benzidine) of 1 grain for 10 ml of DAB buffer for 20 min. Wash 3 times with 2 ml of sterile PBS for 5 min. Wash with 2 ml of sterile distilled water for 5 min. Then, each well was counterstained with 1 ml of Meyer hematoxylin for 10 min. Wash with tap water. Place on glass slides and covered with cover slips.

2.5. Scoring of the VE-cadherin structure

VE-cadherin was observed by the use of a scoring system in view of the utilization of a binocular microscope. Each 100 endothelial cells was subjected to scoring of the structure of VE-cadherin that resembled threads interconnecting ECs in the periphery. Scoring was repeated three times. A score of 2 was given when the observation indicated that 50% or more of the structure of VE-cadherin of ECs were intact. A score of 1 was given when the observation indicated that 50% or more of the structure of VE-cadherin of ECs were broken on the area around the center. Otherwise, a score of 0 was given when the observation indicated that 50% or more of the structure of VE-cadherin of ECs were entirely broken.

3. Results

3.1. Effect of glucose and shear stress on the morphology of endothelial cells

As shown in Fig. 2, exposure to 5 mM of glucose for 7 days prior to treatment with shear stress resulted in ECs having regularly arranged cobble stone-like shape (fusiform). Subsequent to treatment with shear stress for 5 min there were considerable morphological changes in which ECs were irregularly arranged, polygonal, and elongated, and exhibited a decreased ratio of nuclei to cytoplasms. These changes did not occur to the entire ECs, some of them still had fusiform shape. Almost all the entire ECs exhibited changes on minute 8. They were more elongated with nuclei more flattened and the ratio of nuclei to cytoplasms decreased to a greater extent in comparison with that of the treatment with shear stress for 5 min. It appeared that some ECs were already dead, detaching from the base. On minute 12 of the exposure, it was obvious that ECs were even more elongated and lined up according to the direction of shear stress. The most severe damage to the cells was observed on minute 15.

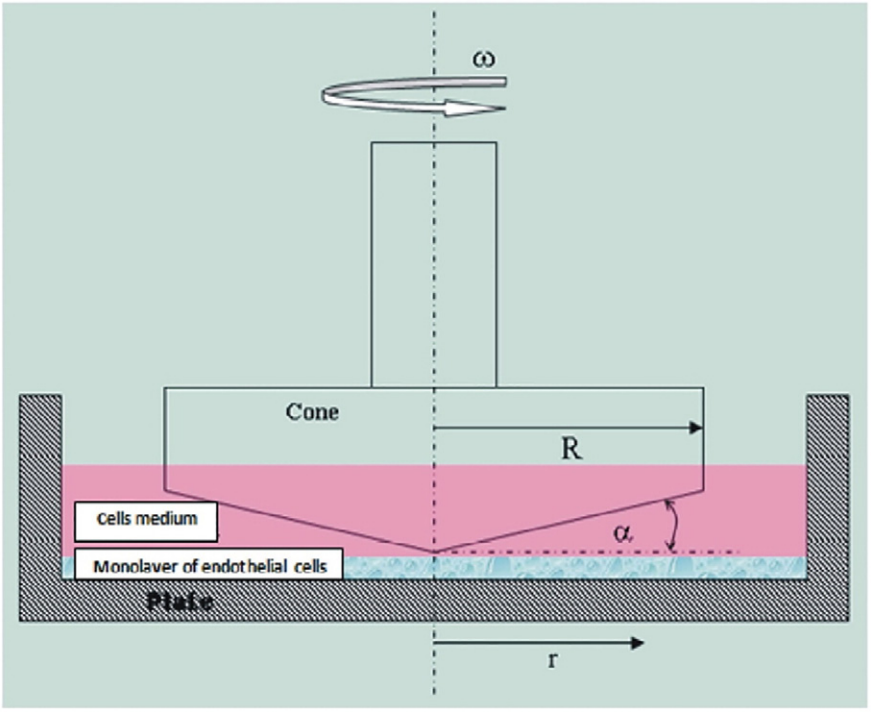

Fig. 1. The design of equipment for inducing shear stress on endothelial cells culture.