Shear Stress Analysis of Synthesis and Nitric Oxide Release from Huvecs Exposed to Supraphysiologic Glucose

Shear Stress Analysis of Synthesis and Nitric Oxide Release from Huvecs Exposed to Supraphysiologic Glucose

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AbstractThe purpose of this study is to analyze the magnitude and duration of shear stress in normal endothelial cell (HUVECs) cultures and those exposed to supraphicological glucose which affected synthesis and release of NO detected by bioassay techniques.The results of this study concluded that large shear stress applied to endothelial cell culture for a long time (± 15 minutes) will increase the synthesis and release of NO. In normal endothelial cell culture a large shear stress exposure in a long time can increase NO synthesis and release very significantly compared to low shear stress (6 dyne/cm^2 for 5 minutes). Likewise, endothelial cell culture which was exposed to 22 mM glucose for 7 days, giving a large amount of shear stress for a long time (15 minutes) could increase NO synthesis and release, although it was far compared to normal endothelial cell culture. It can be said that the synthesis and release of NO in endothelial dysfunction can be improved through exposure to large and long-standing shear stress.

Keywords: shear stress, NO synthesis & release, HUVECs, bioassay techniques

1. Introduction

The main components of blood vessels are endothelial and smooth muscle. Initially, it is known that endothelial function as a barrier system, and blood vessels are considered as pipes that drain blood from the heart to the body organs and tissues. Separate vascular experiments, with and without endothelium, found that acetylcholine causes relaxation of intact blood vessels, but does not affect blood vessels without endothelium [1]. Thus, on this basis it is assumed that acetylcholine stimulates the endothelium to synthesize and release a substance called endothelium derived relaxing factor (EDRF), then it is proven that EDRF is nitric oxide (NO) [2]. NO in endothelial cells serves to maintain basal vascular tone, inhibits leukocyte adhesion, inhibits platelet adhesion, activation, secretion, and aggregation, increases platelet disaggregation, inhibits smooth muscle cell migration and proliferation.

Vascular endothelial cells are located between the walls of vessels and blood, so the endothelium is directly affected by hemodynamic forces such as shear stress, strain and pressure. Thus, the structure and function of synthesis and secretion of vascular endothelial cells is affected by shear stress, namely the force caused by blood flow acting on the cell surface. Shear stress imposed on the Human Umbilical Vein Endothelial Cells (HUVECs) culture regulates the increase of two potential vasoactive endothelium-derived mediators namely vasodilator nitric oxide (NO) and vasoconstrictor endothelin-1

(ET1). So an increase in flow rate through endothelial cells is thought to cause increased release of NO. Likewise with the time (long duration) endothelial exposed to shear stress affects NO synthesis, i.e. the longer the endothelium is exposed to shear stress, the greater the synthesis and release of NO. In addition, the presence of shear stress will cause the diameter of the arteries to change according to the balance of physiological conditions. It can be said that endothelial cells are biosensors of fluid shear stress dynamics that reduce arterial diameter when blood flow decreases and the diameter enlarges when blood flow increases [3].

In the case of diabetes, there is an increase in high blood glucose levels in endothelial cells. This increase will cause changes in glucose's ability to bind proteins, through protein glycosylation processes (non-enzymatic) (Brownlee, 1991). Proteins undergo glycosylation and produce irreversible advance glycosylated end products (AGEs). AGEs are responsible for changes in cell function and produce free radicals that cause cross links and cell proliferation. The main targets of protein glycosylation include the vascular basement membrane cells, because proteins and collagen in tissues have a slow turnover [4]. These changes are related to a decrease in NO function.

Shear stress is not only to determine the ability of blood vessels to release vasoactive substances, but also involved in vascular remodeling and vascular pathobiology (hypertension) [5]. In addition, research that has been conducted at the Biomedical/ Pharmacology Laboratory of the Medical Faculty in Universitas Brawijaya, which sees the effect of supraphysiological glucose levels exposed to endothelial cell cultures from the maternal umbilical vein (HUVECs), to decrease nitric oxide (NO) production. NO released by endothelial cell culture after being induced with Adenosine Diphosphate (ADP) medium was detected by bioassay technique using rat aorta which endothelium had been removed [6]. From this study it was concluded that supraphysiological glucose exposure increased oxidative stress which resulted in the new homeostatasis occurrence in endothelial cells [7]. It is assumed that new homeostasis in endothelial cells causes the synthesis process and release of mediators both from proteins, lipids or other molecules such as NO, thus causing changes in homeostasis in tissues including vascular tissue. Likewise, new homeostasis will give rise to a membrane protein that functions to increase adhesion to blood cells and bacteria to cause an inflammatory process that also underlies macroangiopathic complications (atherosclerosis).

Endothelium location in blood vessels is very beneficial but also at the same time detrimental, because in the hypertension state, diabetes mellitus and endothelial hyperipidemia are targeted (target organs) from damage caused by the disease [8]. Endothelial dysfunction because of high glucose will cause a decrease in NO function. With the decrease in NO function, it causes a decrease in vascular smooth muscle relaxation [9]. The study was reinforced by the existence of research [6], with endothelial dysfunction due to supraphysiological glucose exposure in HUVECs cell cultures causing a decrease in NO release measured by a decrease in rat aortic contractility in which endothelium had been removed.

Many studies have revealed the relationship of hemodynamic (shear stress) and diabetes conditions to the nitric oxide release (NO). Among them state that Glycated low-density lipoprotein inhibits shear stress activating L-arginine in endothelial cells, thereby reducing NO bioactivity [10], whereas a shear stress imposed on hypercholesterolemia patients increases NO release [11]. This is reinforced that aerobic training can improve endothelium-dependent arterial dilation in patients with Impaired Fasting Glucosa (IFG) [12].

Although shear stress conditions regulate the increase in vasodilators of nitric oxide (NO) and high glucose levels reduce the synthesis of NO. As well as glycated low-density lipoprotein inhibits shear stress activating L-arginine which implies a decrease in NO synthesis in HUVECs, but "how the effect of shear stress" on NO synthesis and release of HUVECs exposed to supraphysiological glucose has not been explained.

The study of mechanical biology reveals various mechanical interactions in the endothelial cell life activity to produce various substances. Shear stress has a direct role in the endothelial response.

Through mechanical biology knowledge it is possible to reveal the mechanism of the shear stress influence on nitric oxide (NO) synthesis and release on normal endothelial cell cultures and supraphysiological glucose exposed detected with a potential difference in solution which is further verified by bioassay techniques in the aorta which endothelium has been removed. Shear stress in this study generated cone plates [13] which were driven by an electric motor. The electric motor rotation was regulated by a resistance variable (VR) which was connected to the power supply. By adjusting the speed and angle of the cone plate various shear stresses were obtained, and the cone plate speed was identical to the change in blood vessel flow velocity.

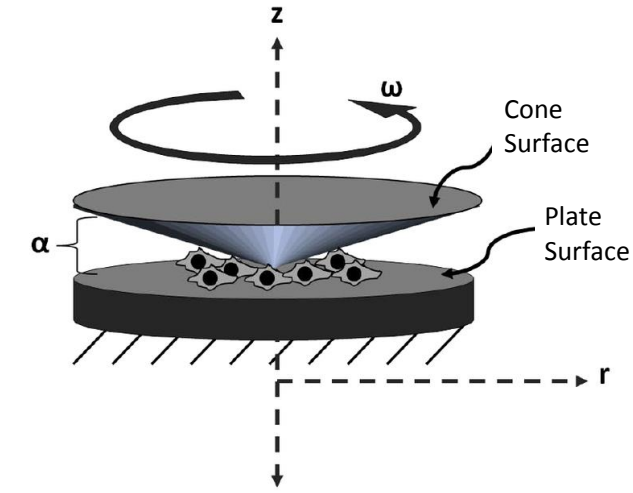

Figure 1. Cone- plate shear stress generator

2. Method

In this study the measurement of nitric oxide synthesis and release using bioassay techniques. The steps were as follows:

1. Administering 2 grams load on the aorta strip in an isotonic condition
2. Dosing of Phenylephrine (PE) 10^{-4} M in 10 m of bath organ
3. Shear stress treatment of 6 & 10 dyne/cm^2 and 5 & 15 minutes at endothelium
4. Administering 22 mM endothelial glucose for 7 days (arjita, et. Al (2001)

Determination of shear stress with cone plate

Flow profile analysis is explained by Newtonian fluid theory and the Stoke Naviers equation. Assuming a flat plat is taken with a cone angle of 5^o (0.08 radians) or less. This device can produce laminar and turbulent flow. Flow can be explained by dimensionless parameters.

$$R\sim = \frac{r^2 \omega \alpha^2}{12v} \quad (1)$$

Where:

r = radial distance from the cone center
ω = angular velocity
α = cone angle (radians)
v = kinematic viscosity

3. Results and Discussions

3.1 Nitric Oxide (NO) synthesis and release graph analysis

The data analyzed were the number of NO syntheses and release calculated from the decrease results of the aortic rats smooth muscle contraction without endothelial obtained from bioassay techniques. The data obtained is shown in the following graphic form Figure 2. Synthesis and release of nitric

oxide in normal endothelium & exposure to 22 mM glucose for 7 days and shear stress treatment 6 & 10 dyne/cm^2 for 5 & 15 minutes Figure 2

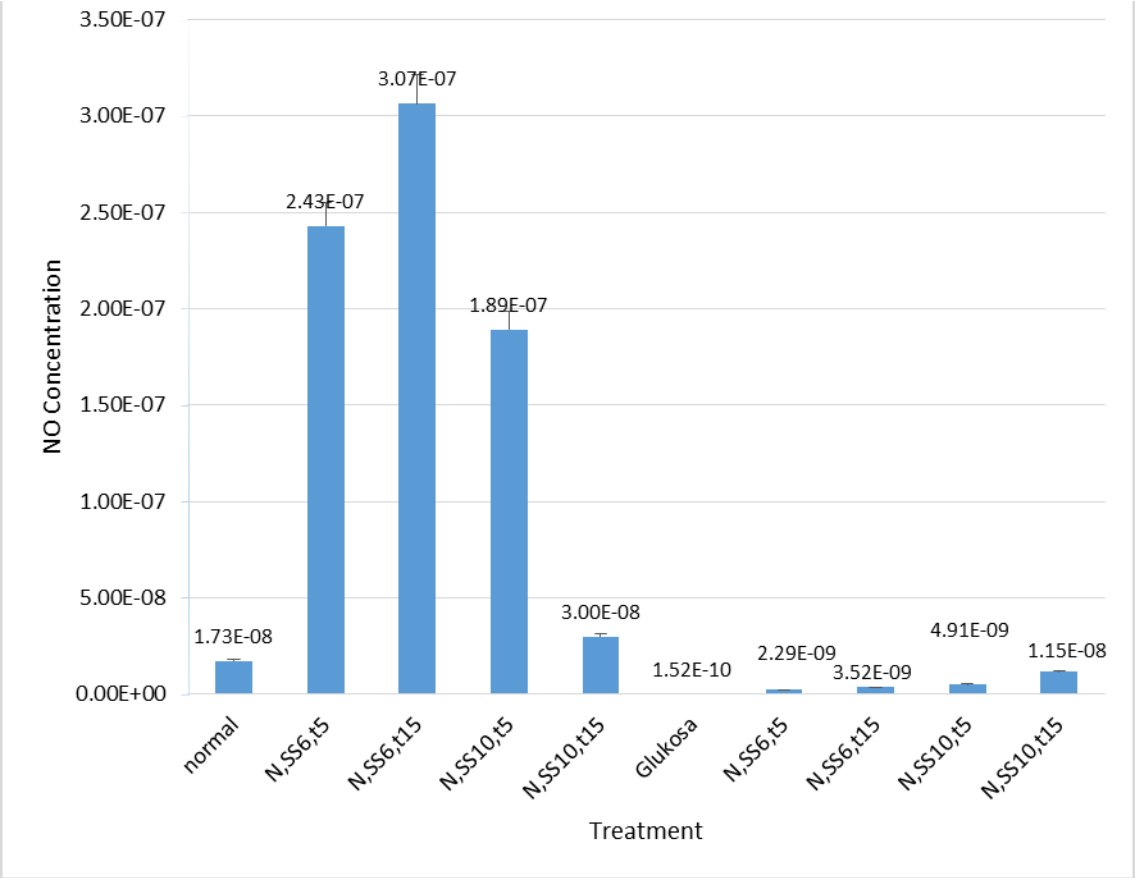

Figure 2. Synthesis and release of nitric oxide (NO) in normal endothelium & exposure to 22 mM glucose for 7 days and shear stress treatment 6 & 10 dyne/cm^2 for 5 & 15 minutes.

In Figure 2, the stem image shows the nitric oxide synthesis and release average value of endothelial cell culture from HUVECs as measured by a decrease in aortic contractility which endothelium was removed after normal administration of phenilephrine 10 & exposed to 22 mM glucose 7 days later treated with shear stress 6 and 10 dyne/cm^2 for 5 and 15 minutes. From the 2-way variance analysis test, it was found that the shear stress treatment affected the synthesis and release of nitric oxide endothelial cell cultures from HUVECs under normal conditions & exposed to 22mM glucose for 7 days with a probability level of 0.2709% ($p < 0.05$). Because the level of probability value is p-0.279% <5%, then the treatment produces significantly different values of nitric oxide (NO) synthesis and release (meaning 99.7291%).

Synthesis and release of nitric oxide (NO) in normal endothelium which is then treated with shear stress 6 & 10 dyne/cm^2 for 5 & 15 minutes Figure 3. In Figure 3, the stem image shows the average value of oxide (NO) synthesis and release endothelial cell culture from HUVECs which is measured by a large decrease in aortic contractility in which the endothel was removed after 10-6 M phenylephrine (PE) under normal conditions exposed to shear stress 6 and 10 dyne/cm* for 5 and 15 minutes. From the analysis of the 1-way variant, it was found that the shear stress treatment affected the synthesis and release of nitric oxide (NO) in endothelial cell cultures of HUVECs under normal conditions with a probability level of 0.780% ($p < 0.05$). Because the level of probability value p

0.780% <5%, the shear stress treatment produces a significantly different average value of nitric oxide (NO) synthesis and release (meaning 99.22%).

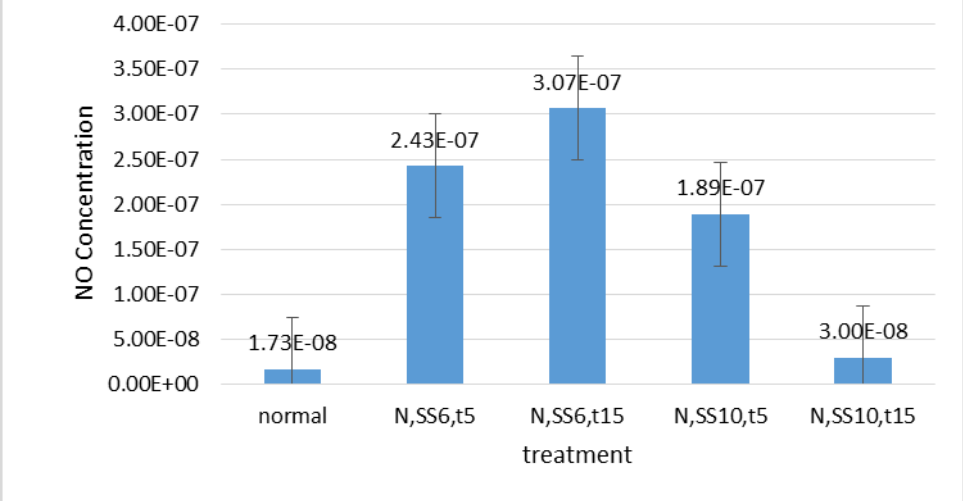

Figure 3. Synthesis and release of nitric oxide (NO) in normal endothelium which is then treated with shear stress 6 & 10 dyne/cm^2 5 & 15 minutes.

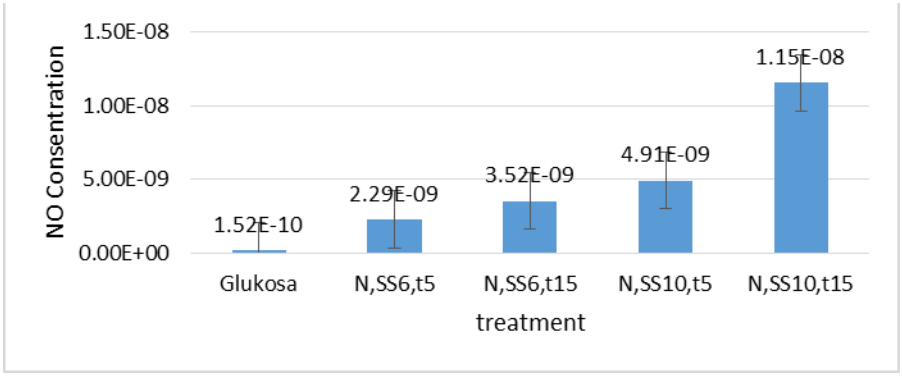

Figure 4. Synthesis and release of nitric oxide (NO) in endothelium which is exposed to glucose 22 mM 7 days and shear stress 6 & 10 dyne/cm^2 for 5 & 15 minutes.

Synthesis and release of nitric oxide (NO) in endothelium which is exposed to glucose 22 mM 7 days and shear stress 6 & 10 dyne/cm2 for 5 & 15 minutes Figure 4. The stem image shows the average value of the synthesis and release of nitric oxide endotrel cell culture from HUVECs which was measured by a large decrease in aortic contractility which the endothelium was removed after phenylephrine which was exposed to 22 mM glucose for 7 days which was then treated with shear stress 6 and 10 dyne/cm^2 for 5 and 15 minutes. With a 1-way variance analysis, it was found that shear stress treatment influenced the synthesis and release of nitric oxide (NO) in endothelial cell cultures of HUVECs which were exposed to 22 mM glucose for 7 days with a probability level of 0.139% (p 0.05). Because the level of probability value is p-0.139% <5%, the shear stress treatment produces significantly different values of nitric oxide (NO) synthesis and release (meaning 99.821%).

Bioassay technique is a method of measuring chemicals that can affect biological elements. In normal endothelial conditions when endothelial cells are stimulated by neotransmitters, certain hormone of hemodynamic forces fiber (shear stress) will cause smooth muscle relaxation in blood vessels which act as a mediator in response to these stimuli is a simple inorganic molecule that easily diffuses

severely when only a few seconds which is none other than endothelium derived relaxing factor and later identified as nitric oxide free radicals [4].

The involvement of normal endothelial cell cultures treated with shear stress on the relaxation of rat aorta which the endothelium has been removed, itcan be identified through the following mechanism: In normal endothelial cell cultures (HUVECs) that are treated with a hemodynamic force (shear stress), a stimulus occurs that activates receptors that are specific to shear stress. This condition will cause the opening of the Ca-ion channel followed by the entry of Ca ions into the intra-cellular cytoplasm. This will lead to interaction of Ca ions with calmodulin which will stimulate the activity of the enzyme endothelium-nitric oxide synthase (e-NOS) as a catalyst in nitric oxide formation reactions. Furthermore, this NO will easily penetrate into the vascular smooth muscle (in this study is the aorta) and works without the need for a receptor but rather the speed of synthesis and release. The presence of NO in smooth muscle will stimulate the formation of cyclic-Guanosine Mono Phosphate (c-GMP) from Guanosine Triphosphate (GTP) which is followed by ion-Ca decreased. The ion-Ca decrease of smooth muscle intracellular will inhibit contractile protein through resistance to the protein Kinase C enzyme and this condition subsequently results in the relaxation of the aortic vascular smooth muscle. Based on previous research it was known that shear stress above 6 dyne/cm^2 can mediate the release of atheroprotective factors from endothelial cells which can inhibit coagulation of leukocyte migration and smooth muscle proliferation. Shear stress above 6 dyne/cm^2 is usually found in arteries in the disebyt Mean Positive shear stress (MPSS) [14] [15] [16]. Large and fast-acting shear stress causes degeneration and erosion of the endothelial surface, while large and long shear stress is supported by small temporal fluctuation [17] [18] mediating atheroprotecyive phenotype. So it can be said that shear stress can affect blood vessel endothelium to stimulate NO synthesis in the endothelium, then catalyze NO formation from arginine. Where NO will stimulate and maintain vasodilation through a chain of biological events that reaches its peak in relaxing smooth muscle cells lining the arteries.

Endothelium which is exposed to glucose 22 mM for 7 days, based on research [1] it was found that endothelium exposed to glucose would reduce NO synthesis and release. This is due to the condition that high glucose levels trigger oxidative stress through auto oxidation pathways, sorbitol myo-inositol and non-enzymatic glycation that have a significant effect on NO synthesis and release. This is apparent in normal endothelial treatment and those exposed to 22 mM glucose for 7 days, both those exposed to shear stress or no reduction in synthesis and release of NO.

The results of the graph 4 show that shear stress affects the synthesis and release of NO in endothelial cell culture which was exposed to 22 mM glucose for 7 days which was observed through aortic contractility which the endothelium had removed. Figure 4 shows the greater shear stress given to the endothelium which was exposed to 22 mM glucose for 7 days increasing synthesis and release of NO. This is supported by [14] [15] [16] which proves that hemodynamic forces (shear stress) can improve endothelium - independent arterial dilation in patients with impaired fasting glucose (IFG). Likewise [11] proves that shear stress can increase endothelium dependent NO-mediated vasodilation from hypercholesterolemic microvasculature.

4. Conclusions

1. Shear stress in endothelial cell cultures (HUVECs) can be generated with cone plate device
2. Large and long shear stress in normal cell cultures can increase NO synthesis and release
3. The magnitude and duration of shear stress in cell cultures exposed to 22 mM glucose for 7 days can improve NO synthesis and release

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