BIOCHEMICAL MEDIATED GLYCOCALYX MODULATION IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVEC)

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INTRODUCTION

The American Heart Association reports that approximately 50,000,000 Americans have high blood pressure, a risk factor for cardiovascular disease and atherosclerosis. Therefore it is necessary to determine the mechanisms involved in the formation of cardiovascular disease. The presence of endothelial cells at the interface of blood flow and the arterial lumen suggests that many factors affecting arterial health are likely to be mediated by the endothelial layer. One way in which endothelial cells may modulate a response to the hemodynamic and chemical environment of the arterial system is via the glycocalyx. In disease states this barrier may be modified and/or protein expression patterns in the cells altered, leading to increased permeability and leukocyte attractiveness. Factors known to affect this transformation include hemodynamics, a variety of cytokines, and certain hormones. Therefore, this work focuses upon endothelial cells and seeks to identify factors influencing activation of the endothelial layer. Glycocalyx modifications in endothelial cells activated by a stimulus may provide a partial explanation for alterations in physiology seen in disease prone areas. Specifically Transforming Growth Factor beta-1 (TGF-β_1), a cytokine, and angiotension II, a hormone, will be studied in the context of their relationship to the development of vascular dysfunction and disease. It was shown by Chua et al. [1] that these two biochemicals are linked. Angiotensin II was found to stimulate production of TGF-β_1 in rat-heart endothelial cells.

Angiotensin II.

Hypertensive patients have higher renin concentrations in the blood stream and therefore increased angiotensin II concentrations, which are known to lead to elevated smooth muscle cell proliferation and alteration of extracellular matrix properties. Over the past several years, the renin-angiotensin system has received increased attention as a primary effecter of vascular disease. Of particular interest to the study of atherosclerosis is the finding that local synthesis of angiotensin II is likely of greater importance to the development and propagation of disease than systemic concentrations [2]. Both endothelial and smooth muscle cells are capable of producing angiotensin converting enzyme (ACE) and other proteins involved in the regulation of tissue function [3].

Transforming Growth Factor – Beta (TGF-β).

Nearly every human cell type, including leukocytes, endothelial cells, and smooth muscle cells, is capable of producing TGF-β_1 and has receptors for it [4]. Many diseases have been linked to the amount of TGF-β_1 produced and activated, including cancer, fibrosis, and atherosclerosis. Main regulatory functions of TGF-β_1 are inhibition of cellular proliferation, promotion of cellular differentation, and induction of apoptosis. TGF-β_1 also affects the recruitment of leukocytes in response to injury and controls the activation and proliferation of inflammatory cells.

MATERIALS AND METHODS

To test the hypothesis that angiotension II and/or TGF-β_1 affects endothelial cell physiology, batch cultures of human umbilical vein endothelial cells (HUVEC) were established and exposed to varying concentrations of each respective biochemical. HUVECs were then evaluated on the basis of proteoglycan composition, glycocalyx thickness and charge, and monocyte adhesion.

Glycocalyx and matrix composition.

Glycocalyx composition was analyzed by non-enzymatically detaching the cells, binding fluorescent lectins, and performing flow cytometry. Similarly, cells were grown on gelatin-coated glass slides and analyzed by fluorescence microscopy. The lectins that were bound include Maackia amurensis (MAA), which binds to sialic acids, concanavalin A (conA), used to label mannose-containing N-linked sugar chains, Triticum vulgare (WGA) that binds to sialic acid and N-acetylglucosamine, and Glycine max (soybean) agglutinin (SBA), which binds to N-acetylgalactosamine.

Glycocalyx thickness and charge.

After cultivation, the endothelial monolayer was fixed with cacodylate buffer containing 2.5% glutaraldehyde and 0.34 M terbium chloride and processed for TEM analysis of glycocalyx thickness. Surface charge was determined by binding cationic ferritin to cells and analyzing TEM images or flow cytometry results on the FITC-conjugate.

Monocyte Adhesion.

Mono Mac 6 cells (MM6) were perfused over confluent HUVEC layers at shear rates of 20, 30, 40, and 50 s^{-1}. Adhesion experiments were performed as described by Rinker et al. [5] for determination of monocyte tethering frequencies, rolling velocities, and adhesion kinetics.

PRELIMINARY RESULTS

Fluorescence microscopy and flow cytometry were used to determine the effect of angiotensin II on glycocalyx composition, and both techniques gave similar results for cells bound with FITC-labeled WGA lectin. Flow cytometry results presented in Figure 1 show an increase in the mean fluorescence intensity as angiotensin II concentration was increased. Similarly, cells grown on glass slides, bound with FITC-WGA and imaged with the fluorescence microscope showed an increase in fluorescence intensity as indicated by a decrease in mean gray value acquired using NIH Image. Other lectins were used to follow glycocalyx changes with angiotensin II. Concanavalin A and MAA binding decreased with increasing angiotensin II levels indicating a decrease in glucose and mannose and sialic acid residues, respectively. The effect of angiotensin on endothelial surface charge was determined with flow cytometry by binding cationic ferritin derivatized with FITC to the cells. Further investigations are planned to clarify the effect on angiotensin II on glycocalyx composition and charge.

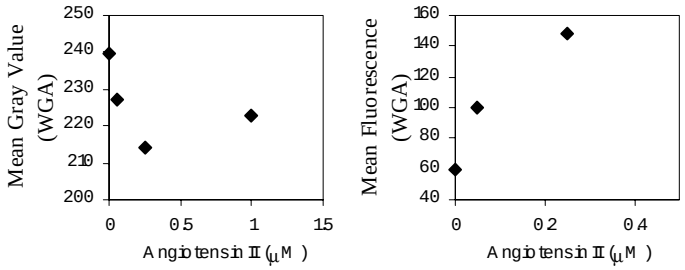

Figure 1. Comparison of fluorescence microscopy and flow cytometry for FITC-WGA lectin binding

Lectin flow cytometry experiments were also conducted to determine the effect of TGF-β_1. FITC conjugates of cationic ferritin and the lectins ConA and MAA were bound to HUVEC cultivated under the following conditions: 1) untreated, 2) exposed for 4 hours to 100 units/mL TNF-α, 3) exposed for 24 hours to 0.8 ng/ml TGF-β_1, and 4) exposed to both TNF-α and TGF-β_1 for the times and concentrations indicated in (2) and (3). Flow cytometry analysis was performed on duplicate samples. For each condition, mean fluorescence intensities +/- standard error of the mean are shown in Table 1. A 24% increase in ConA binding, indicating more glucose and mannose residues, was found for HUVEC exposed to TGF-β_1. However, this result was statistically insignificant due to large sample deviation. Significant increases in MAA binding were found for TNF-α and TGF-β_1 treatments indicating an increase in sialic acid expression under both conditions. The net negative charge of HUVEC treated with TNF-α increased compared to the control possibly indicating the presence of more negatively charged glycoprotein components.

Table 1. Mean fluorescence intensities of HUVEC bound with FITC Conjugates.

Preparation	No Treatment	TNF-α	TGF-β_1	TNF-α & TGF-β
ConA	93.9 ± 12.1	96.4±6.6	116.7±28.5	118.6±17.2
MAA	67.6±3.5	79.3±6.0	75.8±2.6	63.5±13.3
CF	139.8±1.6	145.5±1.2	137.6±4.1	138.0±14.0

Flow experiments were conducted to determine the effect of TGF-β_1 on monocyte adhesion. HUVEC cells were dosed with TNF-α and varying concentrations of TGF-β_1. TGF-β_1 decreased the tethering frequency of MM6 cells on HUVEC while increasing the MM6 rolling velocity as depicted in Figure 2.

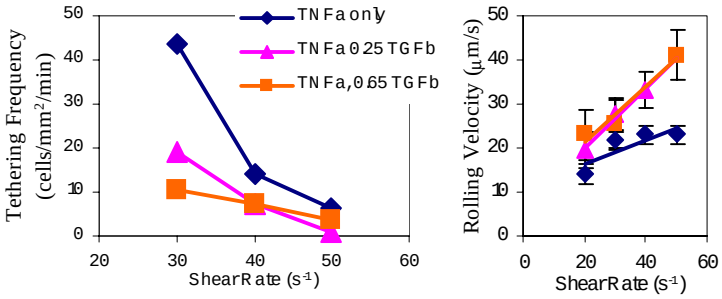

Figure 2. Monocyte Adhesion Study Results

CONCLUSIONS AND FUTURE WORK

From these initial experiments and results, both angiotensin II and TGF-β_1 do appear to have an effect on endothelial cells. Further experiments are underway to clarify their effect on glycocalyx composition and charge. Experiments are also planned to more accurately represent in vivo conditions. A continuous perfusion apparatus has been developed that allows for the long-term cultivation of HUVECs under pulsatile flow conditions. The batch culture experiments will be repeated for cells grown under pulsatile flow to examine differences in static and dynamic culture environments on cell phenotype.

REFERENCES

1. Chua, C. C., Diglio, C. A., Siu, B. B., and Chua, B. H. L., 1994, "Angiotensin-II Induces TGF-Beta-1 Production in Rat-Heart Endothelial Cells," Biochimica et Biophysica Acta – Molecular Cell Research, Vol. 1223, pp. 141-147.
2. Weiss, D., Sorescu, D., and Taylor, W. R., 2001, "Angiotensin II and Atherosclerosis," Am. J. Cardiol. Vol. 87, pp. 25C-32C.
3. Dzau, V. J., 1988, "Circulating Versus Local Renin-Angiotensin System in Cardiovascular Homeostasis," Circulation, Vol. 77, pp. 1-4.
4. Blobe, G. C., Gerard, C., Schiemann, W. P., and Lodish, H. F., 2000, "Mechansims of Disease: Role of Transforming Growth Factor (beta) in Human Disease," N. Engl. J. Med. Vol. 342, pp.1350-1358.
5. Rinker, K. D., Prabakhar, V., and Truskey, G. A., 2001, "Effect of Contact Time and Force on Monocyte Adhesion to Vascular Endothelium," Biophys. J., Vol. 80, pp. 1722-1732.