HEPARAN SULFATE PROTEOGYLCAN IS A MECHANOSENSOR ON ENDOTHELIAL CELLS

Jeffry A. Florian, Jason Kosky, Kristy Ainslie, Zhengyu Pang, Randal O. Dull*, John M. Tarbell

Biomolecular Transport Dynamics Laboratory, Departments of Chemical Engineering and Bioengineering
The Pennsylvania State University
University Park, PA 16802
*Department of Anesthesiology and Critical Care Medicine
The Johns Hopkins University School of Medicine
Baltimore, MD

INTRODUCTION

The inner surfaces of blood vessels are lined with a monolayer of endothelial cells (ECs) that is continually exposed to the mechanical shearing forces (stresses) of blood flow. The exposure of endothelial cells to shear (both steady and oscillatory) has been shown to alter the production of vasoregulating agents of which nitric oxide (NO) and prostacyclin (PGI_2) are perhaps the most notable. NO and PGI_2 responses to shear stress are mediated by mechanotransduction or the transmission of the fluid shear force to the cell and its transduction into a biomolecular response. The hypothesis of this study is that the plasma membrane surface layer, termed the glycocalyx, is the primary mechanosensor for the NO and PGI_2 responses.

The surface of endothelial cells is decorated with a wide variety of membrane-bound macromolecules including glycoproteins and proteoglycans that constitute the glycocalyx. Proteoglycans can be divided into different groups based on their protein backbones (e.g., syndecans and glypicans). Glypicans form the second most common proteoglycan group, and possess structural similarity, typically differing only in the number of glycosaminoglycan (GAG) attachment sites. These molecules are bound to the surface through a COOH-terminal GPI (glycosyl phosphatidyl inositol) anchor (1). Syndecans form the largest group of proteoglycans on the endothelial surface and are distinguished by highly variable extracellular domains (2) and by their status as the only proteoglycan that penetrates the cytoplasm, allowing for interaction with the cytoskeleton (3). Heparan sulfate is the most common GAG, comprising 50-90% of the GAGs on the endothelial cell surface (4).

In order to assess the role of the glycocalyx in shear-induced NO and PGI_2 production, we employed an engineered shearing device to expose monolayers of bovine aortic endothelial cells (BAECs) to defined shear stress (steady and oscillatory). Heparinase III was used to selectively deplete the glycocalyx of the heparan sulfate GAG component. Then NO and PGI_2 production rates in response to shearing conditions were determined for heparanase treated and untreated monolayers.

METHODS

The heparan sulfate degrading enzyme, heparinase III from Flavobacterium heparanum, and bradykinin (BK) were obtained from Sigma, and the heparan sulfate primary antibody, HepSS-1, was obtained from US Biologicals. BAECs were harvested from thoracic aortas as described by Sill et al. (5) and expanded using standard cell culture techniques. Cells (passage 5-9) were seeded onto fibronectin-coated slides and used in experiments 3-4 days after cell seeding. Heparinase treatment involved incubation of the BAEC monolayer with enzyme at a concentration of 15 mU/ml for 2 hours followed by washing with experimental medium (2x).

A rotating cylindrical disk shear apparatus that has been described previously (6) was used to impose either steady or oscillatory shear patterns. In some experiments BK was added to the experimental media (5 nM) to stimulate NO production without mechanical stimulation. NO concentration was quantified as NO_2^- using nitrate reductase and a fluorimetric assay. PGI_2 was quantified using a commercial EIA system (Amersham).

RESULTS

To verify the removal of heparan sulfate by the heparinase treatment, the fluorescence of a secondary antibody linked to the HepSS-1 primary antibody was quantified. After heparinase treatment, there was 45 % reduction in fluorescence intensity indicating that the enzyme was effective in removing heparan sulfate.

Cells treated or untreated with heparinase were exposed to a step change from 0 to 20 dyn/cm^2 steady shear stress and NO cumulative concentrations in the media were determined at time intervals for 2 hours. Stationary controls with and without heparinase were run concurrently. The 2 hour NO concentrations will be reported here as a measure of production rate in units of nmol/million cells.

Control:	2.22 +/- 0.23 (mean +/- SEM)
Control with heparinase:	1.67 +/- 0.40
Steady shear:	6.54 +/- 0.42

Steady shear with heparinase: 2.56 +/- 0.22

Similar experiments were run under oscillatory shear conditions with a mean component of 10 dyn/cm^2 and an amplitude of 15 dyn/cm^2 resulting in a waveform with reversal of shear stress direction over part of the cycle. The 2 hour NO concentrations in the same format as above were:

Oscillatory shear:	9.07 +/- 0.60
Osc. shear with heparinase:	2.61 +/- 0.56

Experiments run under stationary conditions but with bradykinin pre-treatment led to the following 2 hour NO concentrations:

Bradykinin:	6.97 +/- 0.55
Bradykinin with heparinase:	7.25 +/- 0.57

The results above indicate that heparinase greatly inhibits both steady and oscillatory shear-induced NO production and that bradykinin-induced NO production is unaffected by heparinase. To test whether heparan sulfate GAG is a more general mechanosensor, we measured PGI_2 concentration over 2 hours of steady shear stress at 20 dyn/cm^2 for heparinase treated and untreated monolayers. The 2 hour concentration values in normalized units are presented below.

Control:	1.0
Control with heparinase:	5.1 +/- 0.2
Steady shear:	42.5 +/- 5.0
Steady shear with hep:	65.0 +/- 5.5

These results show that heparinase treatment does not inhibit shear-induced PGI_2 production.

DISCUSSION

The results indicate that heparinase greatly inhibits shear-induced NO production without inhibiting the downstream cellular machinery that mediates NO production. This suggests that heparan sulfate is a mechanosensor for NO production. On the other hand, PGI_2 was not inhibited by heparinase indicating that heparan sulfate is not a mechanosensor for PGI_2. These results show that endothelial cells possess more than one primary mechanosensor. It is possible that other GAG components such as chondroitan sulfate serve as specific mechanosensors. It should also be recognized that even if one cell structure that can sense shear stress is removed from the cell surface (such as heparan sulfate), the same level of shear stress must be transmitted to the cell (balance of forces). Other structures on the apical surface, within the cell, or on the basolateral surface can still be mechanically influenced by shear stress, although the primary sensing mechanism may vary.

ACKNOWLEDGEMENTS

This work was supported by NIH NHLBI Grants HL35549 and HL57093 to JMT and HL680683 to ROD.

REFERENCES

1. Wenksberg R, Squire JA, and Templeton DM. Glypicans: a growing trend. Nat. Genet. 12. 225-7. 1996.
2. Bernfield M, Kokenyesi R, Kato M, Hinkes MT, Spring J, Gallo RL, Lose EJ. Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans. Annu Rev Cell Biol. 8. 365-393. 1992.
3. Asundi VK, and Carey DJ. Self-association of N-syndecan (syndecan-3) core protein is mediated by a novel structural motif in the transmembrane domain and ectodomain flanking region. J Biol Chem. 270. 26404-10. 1995.
4. Ihreke NS, Wrenshall LE, Lindman BJ, and Platt JL. Role of heparan sulfate in immune system-blood vessel interactions. Immunol Today. 14. 500-5. 1993.
5. Sill H, Butler C, Hollis T, and Tarbell J. Albumin permeability and electrical conductivity as means of assessing endothelial cell monolayer integrity. J Tissue Culture Methods. 14. 253-8. 1992.
6. Sill HW, Chang YS, Artman JR, Frangos JA, Hollis TM and Tarbell JM. Shear stress increases hydraulic conductivity of cultured endothelial monolayers. Am J Physiol. 268. H535-43. 1995.