Original Article

Hemodynamic Forces Induce the Release of Nitric Oxide from Aortic Endothelium: Combined Effect of Shear Stress and Pressure

Husain S Yawer[1], Sadik R Panwar[2], Nidhi Priya[3], Ali S[4], Alok R Ray[5]

[1]Deparment of Dental Materials, RUHS College of Dental Sciences, Jaipur, Rajasthan; [2]Consultant, Interventional Cardiology, Beckley ARH Hospital ,Beckley, West Virginia, USA; [4]Assistant Professor. Department of Pathology, RUHS College of Medical Sciences, Jaipur, Rajasthan; [4]Professor and Head, Department of Biochemistry, Jamia Hamdard University, New Delhi, [5]Professor, Centre for Biomedical Engineering, Indian Institute of Technology, New Delhi, India

ABSTRACT

Introduction : Fluid shear stress (pulsatile and laminar) and pressure comprise of the two *in vivo* physical forces to which vascular endothelium is constantly exposed. Besides, vasodilator and autacoids, nitric oxide is the main endothelium derived relaxing factor (EDRF) released in response to alterations in blood pressure and shear stress, which constitute hemodynamic stress.The aim of the present study was to study the effect of pulsatile/laminar shear stress on endothelial nitric oxide production .

Methodology : A perfusion system was used to study the effect of shear stress on endothelial nitic oxide production. It was designed as a close continuous flow loop in which the pressure was superimposed with flow through a pressure head with adjustable height.

Results: At hydrostatic pressure of 70 cm water (normotensive condition) and at 150 cm water (hypertensive condition), an increase in nitric oxide concentration was observed. The increase was 10 fold higher at 150 cm with respect to static values than the increase observed at 70 cm of water.

Conclusion: Laminar shear stress and pressure induce the release of endothelial nitric oxide, which may aid to understand the role of wall shear stress during arterial resistance in hypertension.

INTRODUCTION

Endothelial cells synthesize and secrete compounds that affect many processes: homeostasis such as vascular tone and immune responses.[1,2,3] The hemodynamic forces generated by blood flow act by modulating endothelial cell's synthesis and secretion of bioactive molecules thus transiently regulating vascular tone (short term action). In addition, blood mechanics regulate vascular remodeling as seen in changes in pressure and flow rate (shear stress) during hypertension) during long-term changes.

Endothelial cells have conventionally been recognized to produce and release vasoactive substances to control the vascular tone and peripheral circulation by several mechanical and chemical stimuli.[4] Studies carried out by Harrison et al [5] and Awolesi et al [6] show that production of NO, as well as the expression of eNOS (endothelial Nitric Oxide Synthase) by vascular endothelial cells is dependent on mechanical factors. Shear stress and cyclic circumferential stretch have been demonstrated to increase NO release as well as upregulate eNOS mRNA and protein.[7] In a recent study using an *in vitro* tube model in which pressure, shear stress, and cyclic circumferential stretch was combined, shear stress has been shown to represent the major mechanical factor inducing an increase in eNOS expression in bovine aortic endothelial cells (BAECs) .[8] Pulsatile pressure-induced cyclic stretch, however, did not significantly alter changes in eNOS expression in cells exposed to shear stress. Unlike unidirectional shear stress, oscillatory shear stress did not induce any change in eNOS expression compared with static endothelial cell culture. The inevitability of flow pulsatility in the systemic circulation remains controversial.

Many studies in the past have revealed the beneficial effects of pulsatile systemic blood flow over laminar blood flow on the peripheral circulation, organ function, or metabolism.[9,10] Nevertheless, these mechanisms have yet to be clearly elucidated.

Subjecting vascular endothelial cells to mechanical stresses has been a challenge.[11] In the present study two mechanical forces, shear stress and hydrostatic pressure (70 or 150 cm water, representing normo- and hypertensive situations, respectively) have been combined to determine the differential effect of flow pattern (pulsatile/laminar shear stress) on endothelial nitric oxide production as well as to determine whether hydrostatic pressure in combination with flow has a regulatory function on endothelial cells with respect to nitric oxide production.

METHODS

Animal Model: Thoracic aortas (n=20) of adult male white rabbits (1.0-1.2 kg) were used through out the study. All experiments were performed in accordance with the guidelines of the animal ethics committee of the institute. All animals were fed a commercial pellet diet and given water *ad libitum.*

Perfusion system: A perfusion system was designed (Figure 1) with some modifications in a previously used perfusion system by Baldwin et al.[12] The composition of Krebs solution, represented as mMol/L, was 118 NaCl; 4.7 KCl; 25 $NaHCO_3$; 1.2 $KH2PO_4$; 1.2 $MgSO_4$; 2.5 $CaCl_2$; and 11 glucose was used. The perfusate contained bovine serum albumin (BSA) (Cohn Fraction V, Sigma, St Louis, USA) at a concentration of 10 mg/ml in Krebs solution. Perfusates were warmed to 37 °C and pH adjusted to 7.4, finally it was oxygenated with 95% O_2 + 5% CO_2, prior to perfusion in the vessel. N-nitro L-arginine methyl ester (L-NAME, Sigma) was added at a concentration of 10^{-4} M in the perfusate when required. In the perfusion system, pressure was superimposed with flow through a pressure head with adjustable height, connected upstream to the vessel. The perfusate circulated between downstream and upstream reservoir, through a roller pump (Minipuls, Gilman, USA). All the tubings used in the system were of uniform diameter to maintain proper flow conditions.

For the experiments with pulsatile flow (shear stress) and pressure, a solenoid valve (Nachiketa Fluidomics, India), operated through a switching circuit of 1.6-3.0 Hz was introduced between upstream reservoir and outer vessel bath. A catheter tip pressure transducer (Excel Technologies, India) was attached through the lumen at the downstream end of the aorta. It was positioned as close as possible to the vessel to minimize the pressure drop down the length. Artery preparation and cannulation procedure described by Baldwin et al [12] was followed.

The extent of flow pulsatility was calculated ($\acute{\alpha}$=4.94) using a Womersley approximation.[13] Shear stress was calculated according to Poiseuille's law, according to which a relatively small decreases in vessel diameter at constant flow can markedly increase shear stress at the endothelial surface. The value for wall shear stress was kept (Mean $\pm$ SD) 1.0 $\pm$ 0.05 dyne /cm^2 at 70 or 150 cm water pressure. The perfusate was allowed to circulate for the desired length of time. The design of the cannula ensured a fully developed laminar flow in the artery.

Study involving laminar shear stress and pressure: The flow system and the cannula were designed to provide a steady and uniform laminar flow in the artery. The endothelial nitric oxide was measured in static as well as flow (laminar shear stress) conditions with varying hydrostatic pressures. The artery was pressurized through a fluid filled calibrated capillary tube (internal diameter 1.2 mm) containing an air bubble meniscus. This capillary was connected to a reservoir placed at a height to achieve the desired hydrostatic pressure (Figure 1). In each artery, measurements were made either at 70 cm (n = 5) or 150 cm (n = 5) water pressure. At the start of the experiment, hydraulic flux was measured till steady state (tissue stabilization) was reached. The sequence of measurements was as follows:

(i) Steady state under static conditions (no luminal flow)

(ii) Steady state in presence of L-NAME in the perfusate and under static conditions

(iii) Steady state after removal of perfusate containing L-NAME and no luminal flow (reversibility)

(iv) Steady state after luminal flow is introduced

(v) Steady state in presence of luminal flow and L-NAME in the perfusate

(v) Steady state in presence of perfusate flow without L-NAME in the perfusate (reversibility)

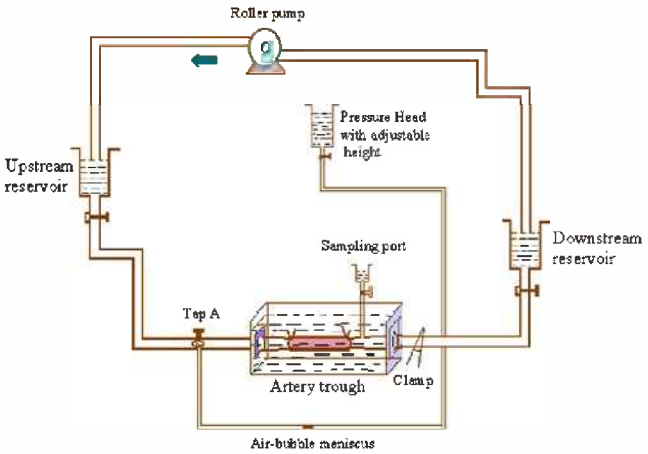

Figure 1: Perfusion system used to impose laminar shear stress and hydrostatic pressure on aorta.

The purpose of using L-NAME was to confirm whether vascular endothelium is actively producing nitric oxide with respect to shear stress and pressure.

Studies involving pulsatile shear stress and pressure :

The endothelial nitric oxide was also measured in conditions of pulsatile flow (1.6 Hz) and varying hydrostatic pressure in normo-tensive and hypertensive conditions at the onset of flow (0 min) and after steady/constant flow state under pulsatile flow conditions (60 min) (Figure 2).

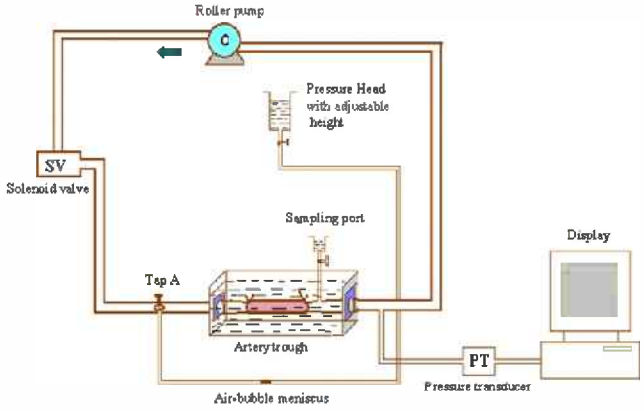

Figure 2: Perfusion system used to impose pulsatile shear stress and hydrostatic pressure on aorta (catheter-tip pressure transducer inserted at the downstream end of vessel).

Flow was induced in the physiological arterial direction by opening stopcock A and the clamp simultaneously. By adjusting the heights of upstream and downstream reservoirs the flow rate was so adjusted that a constant arterial pressure could be maintained and the position of air bubble in the capillary tube 'A' was used to monitor this. The flow was measured by observing the appearance of a fixed volume of perfusate per unit time in the downstream reservoir. The flow rates were (Mean $\pm$ SD) 0.15 ± 0.03 and 0.18 ± 0.08 ml/sec at 70 and 150 cm of water pressure, respectively.

Estimation of nitric oxide: Spectrophotometric estimation of NO at 520 nm was done using a modified version of the previously established method[14], which utilizes Greiss reagent. The concentration of NO (nM/ml) was measured as amount of nitrite present in the sample. Samples (200 l) for measurement were collected at 0, 15, 30, 45, 60 minutes (15 min time interval). Absorbance of each sample was recorded colorimetrically and the concentration of nitrite was expressed as nmol/ml. The calibration curve was prepared using sodium nitrite solution.

RESULTS

The data presented in the study were observed in individual aortas. At 70 cm water, pulsatile flow initially enhances NO production but at steady state (after 60 min) it decreases the level of NO (Figure 3). At higher pressure (150 cm water), the inversed observation was recorded; NO production was higher at steady state (at 60 min) (Figure 4). On comparison of the total amount of nitric oxide produced in pulsatile shear stress and pressure with non-pulsatile or laminar shear stress and pressure, the release was higher in the latter case. Release of NO in case of pulsatile shear and pressure was more at 150 than 70 cm water at 60 minute.

Present study reports that increase in NO production caused by pulsatile shear stress with respect to static state was several fold higher than the same observed at 70 cm H_2O. Further, a decrease in NO concentration in presence of L-NAME when artery was exposed to pulsatile shear stress was lower at 150 cm H_2O than at 70 cm H_2O (Figure 5 and 6). The effect of L-NAME at higher pressure may not be marked because NO production at higher pressures is low as such. The inhibitory effect of L-NAME under no flow conditions did not exhibit any comparable trend between the two pressures. At 150 cm water, in three out of the five experiments, NO concentration was found to be zero (below the detection limit of this method) under static conditions, when L-NAME was present in the perfusate (Figure 6).

Under static conditions, in absence of shear stress (laminar/pulsatile) the concentration of NO was higher at 70 cm H_2O than at 150 cm H_2O (Figure 3 and Figure 4). At

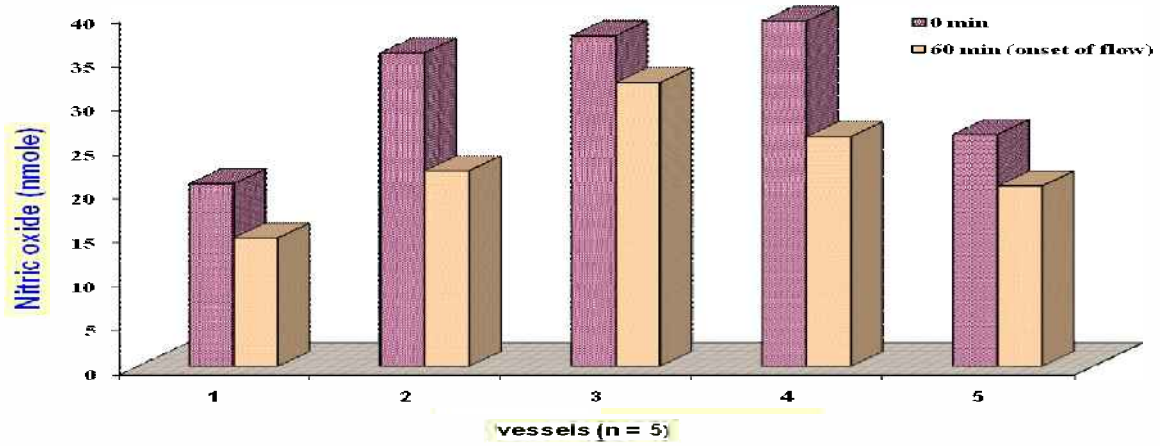

Figure 3: Endothelial nitric oxide production in aorta (n = 5) with respect to pulsatile shear stress.
Each artery pressurized at 70 cm water. Shear stress value in flow conditions : 1.0 + 0.05 dyne/cm2

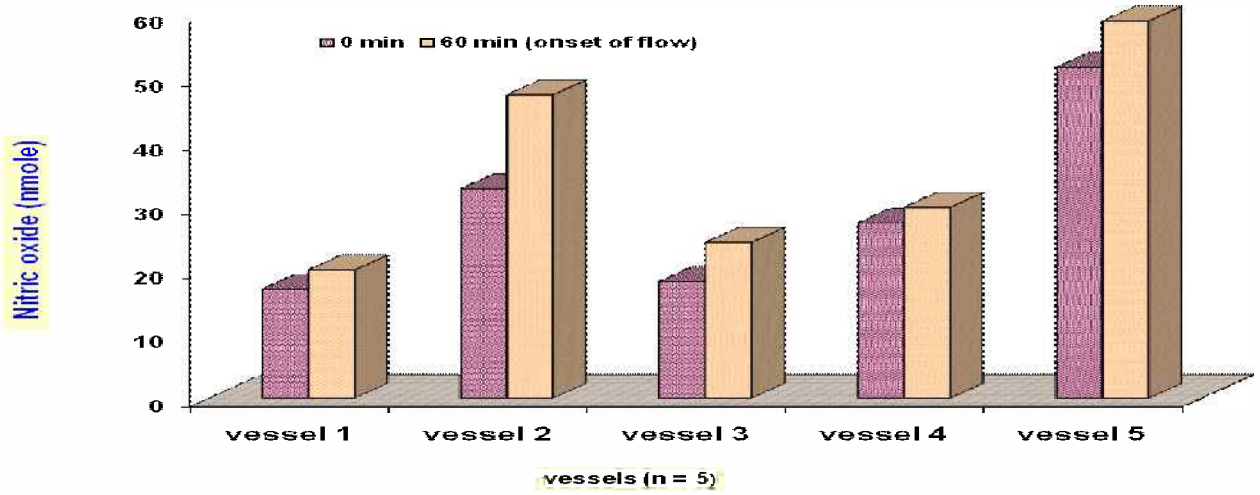

Figure 4: Endothelial nitric oxide production in aorta (n = 5) with respect to pulsatile shear stress.
Each artery pressurized at 150 cm water. Shear stress value in flow conditions : 1.0 + 0.05 dyne/cm2 .

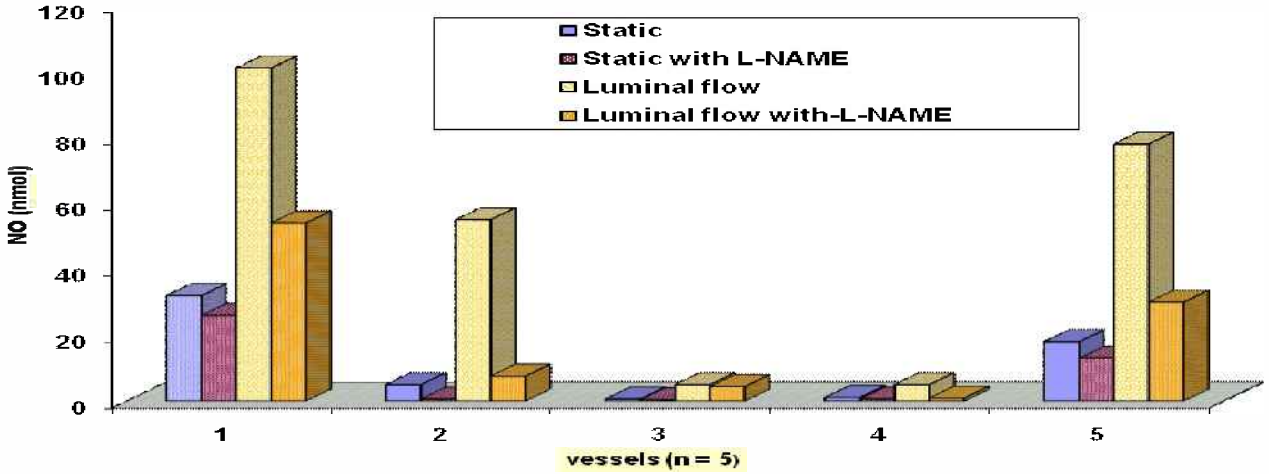

Figure 5: Endothelial nitric oxide production in aorta (n = 5) with respect to laminar shear stress, under different experimental conditions.
Each artery pressurized at 70 cm water. Shear stress value in flow conditions : 1.0 + 0.05 dyne/cm2.

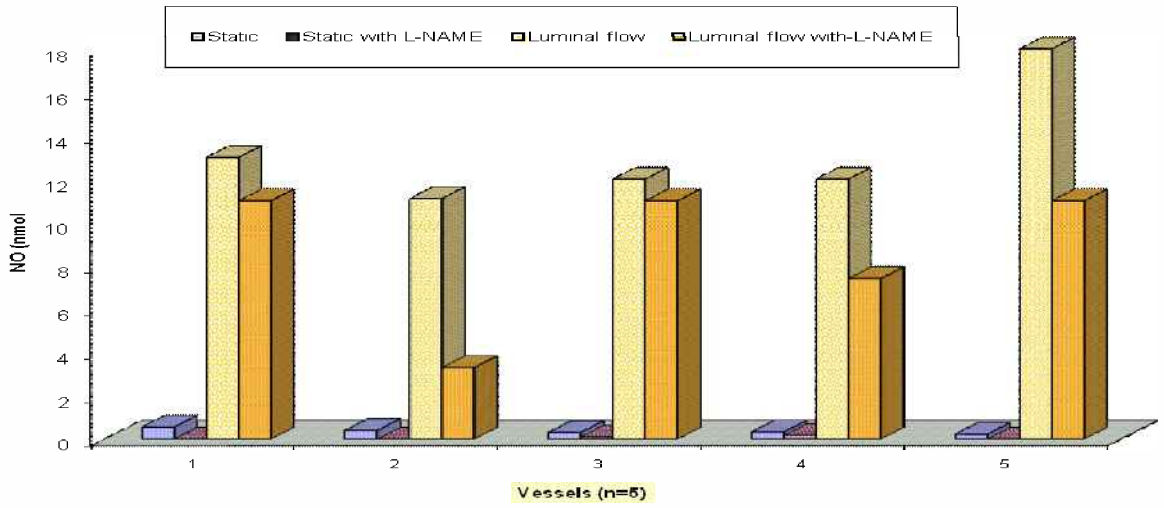

Figure 6: Endothelial nitric oxide production in aorta (n = 5) with respect to laminar shear stress, under different experimental conditions.

Each artery pressurized at 150 cm water. Shear stress value in flow conditions : 1.0 + 0.05 dyne/cm2.

both pressures, the NO concentrations were higher in presence of shear stress (laminar/pulsatile) as compared to static conditions.

DISCUSSION

The series of experiments conducted aimed at determining the degree to which endothelial derived relaxing factor (or nitric oxide) release on stimulation by the spatial pattern (pulsatile/laminar) of hemodynamic forces in normotensive (70 cm water pressure) and hypertensive (150 cm water pressure) states.

Both mechanical stress parameters (*viz.* intraluminal pressure, laminar and pulsatile shear stress) were acting on the endothelium simultaneously, which is a close simulation of the *in vivo* conditions though not an ideal one. The shear stress values were low on account of low flow rates, which had to be maintained in order to keep the transmural pressure constant. In each artery, NO production was measured in presence or absence of luminal flow imposed shear stress as the artery was exposed to intraluminal pressure of either 70 or 150 cm of water. Although, efforts were made to keep all the animals under uniform physiological conditions, the data was highly scattered at 70 cm water indicating that there is high degree of vessel-to-vessel variability at this pressure. The reason for this variable response is still unknown. The values for nitric oxide release were highly statistically significant due to vessel-to-vessel variability[15,16] overall pattern was found to be significant under different experimental conditions.

The results obtained in the present study under static conditions in absence of shear stress support an earlier study done by Baldwin et al [15] in which a depression in NO release at elevated intraluminal pressure was observed. NO synthase activity enhanced by shear stress has been shown in many studies largely pertaining to endothelial cell cultures.[17,18] Studies carried out in isolated rabbit mesentery demonstrated that an increase in transmural pressure under no flow conditions could result in a marked vessel wall contraction. However, when flow was permitted to increase under these experimental conditions, a dilatation was observed.[19] The results of this study are significant in that the experiments were performed on endothelial cells from intact vessels in an *ex-vivo* preparation. It has been studied [20] that in absence of ideal hemodynamic conditions (i.e. normal pulsatility, cyclic strain, and volume flow), endothelial NO production does not accomplish the levels as it occurs under ideal physiological conditions. Pulsatile flow and vessel compliance have a key role in NO production by vascular endothelium in a three-dimensional hemodynamically active model. Several analysis have been made regarding the effects of transmural or hydrostatic pressure on endothelium derived nitric oxide production. Rubanyi et al[21] and Hutcheson et al [22] have studied earlier using a cascade bioassay system, that escalation in the hydrostatic pressure or pulse pressure caused a decrease in NO release at a constant flow rate. However, they could not rule out the possibility that

pressure-induced, endothelium-independent vascular distension might lead to a fall in shear stress on endothelial cells, which consequently may cause a reduction in NO production rather than the direct effect of pressure on the same.

The combination of pressure and shear stress was studied by Kanai et al[23] on the expression of endothelial NO synthase. They imposed a very low level of shear stress and found no significant endothelial NO production. When absolute values of NO were compared between the two pressures, it was found that the values of NO were higher at 70 cm H_2O than at 150 cm H_2O after flow was induced. It is presumed that, this can be attributed to the generation of superoxide anion radical due to enhanced pressure and shear stress.

Superoxide anion quenches nitric oxide by converting it to peroxynitrite, which cannot be estimated by the biochemical assay. When L-NAME is present, nitric oxide is completely absent and superoxide then expresses its effect on transvascular transport. Superoxide enhances the oxidative modification of lipoproteins and other blood components. These cellular responses may promote the process of vascular pathologies like atherosclerosis.

The precise mechanism by which shear stress regulates the production of NO is yet to be further explored. Endothelial responses to flow alterations by means of mechanosensor elements may represent a fundamental physiological paradigm. *In vivo* studies have found that flow reorganizes endothelial cell cytoskeletal proteins.[24] The cytoskeleton may also serve as a second signal apparatus for transducing the biomechanical stimulus sensed by endothelial surface to the nucleus, which would favor transcriptional activation of NO synthase mRNA.[11]

Pulsatile flow has been shown to stimulate the release of endothelium-derived relaxing factors in excess of that occurring with steady flow.[25] But it was later revealed that pulsatile flow alone is a weak inducer of NO. Even though both steady and pulsatile elements of flow are transduced by the endothelium, it is uncertain to explain whether they involve the same or different signal transduction mechanisms. It is speculated that vascular smooth muscle cells may be involved in the production of intracellular nitric oxide, since in response to mechanical injury, smooth muscle cells migrate in the intima and proliferate in contact with the blood flow-induced shear stress.

CONCLUSION

Production of vasoactive substances by vascular endothelial cells is related to the fluid shear stress condition experienced by endothelial cells. These findings may further contribute to our understanding of the physiological role of pulsatile and laminar blood flows. The results emphasize the importance of using a quasiphysiological hemodynamic environment combining all stresses to assess vascular wall function in vitro.

Limitations: In the present study only the effects of acutely imposed pulsatile flow were evaluated, studies are therefore required to consider the change in the vascular responses to long term pulsatile/laminar blood flow.

Acknowledgment: SYH acknowledges CSIR for providing financial assistance for the study in the form of JRF and SRF.

REFERENCES

1. Yazaki, Y. Hemodynamic shear stress stimulates endothelin production by cultured endothelial cells. Biochem Biophys. Res Commun 1989; 161: 859–864.
2. Awolesi, M.A., M.D. Widmann, W.C. Sessa, B.E. Sumpio. Cyclic strain increases endothelial nitric oxide synthase activity. Surgery 1994; 116: 439–444.
3. Chen, H.I., I.P. Chiang, C.J. Jen. Exercise training increases Acetylcholine-stimulated endothelium-derived nitric oxide release in spontaneously hypertensive rats. J Biomed Sci 1996; 3: 454-460.
4. Hecker, M, A. Mulsch, E. Bassenge, R. Busse. Vasoconstriction and increased flow : two principal mechanisms of shear stress-dependent endothelial autacoid release. Am J Physiol 1993; 265: H 828 - H 833.
5. Harrison, D.G., H. Sayegh, Y. Ohara, N. Inoue, R.C. Venema. Regulation of expression of the endothelial cell nitric oxide synthase. Clin Exp Pharmacol Physiol 1996; 23: 251–255.
6. Awolesi, M.A., W.C. Sessa, B.E. Sumpio. Cyclic strain upregulates nitric oxide synthase in cultured bovine aortic endothelial cells. J Clin Invest 1995; 96: 1449-1454.
7. Hishikawa, K., T.F. Lüscher, Pulsatile stretch stimulates superoxide production in human aortic endothelial Cells. Circulation 1997; 96: 3610-3616.
8. Ziegler, T., K. Bouzourene, V.J. Harrison, H.R. Brunner, D. Hayoz. Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthase in cultured endothelial cells. Arterioscler Thromb Vasc Biol 1998; 18: 686–692.
9. Jacobs, L.A., E.H. Klopp, W. Seamone, S.R. Topaz, V.L. Gott. Improved organ function during cardiac bypass with a roller pump to deliver pulsatile flow. J Thorac

Cardiovasc Surg 1969; 58: 703-712.

10. Dunn, J., M.M. Kirsh, J. Harness, M. Carroll, J. Straker, H Sloan. Hemodynamic, metabolic, and hematologic effects of pulsatile cardiopulmonary bypass. J Thorac Cardiovasc Surg 1974; 68: 138-147.
11. Resnick, N., M.A. Gimbrone Jr. Hemodynamic forces are complex regulators of endothelial gene expression. FASEB J 1995; 9: 874–882.
12. A.L. Baldwin, L.M. Wilson, B.R. Simon. Effect of pressure on aortic hydraulic conductance. Arterioscl Thromb 1992; 12: 163-171.
13. H.R. Brunner. Spontaneous diameter oscillations of the radial artery in humans. Am J Physiol 1993; 264: H2080–H2084.
14. Uehera, Y., A. Numabe, S. Takada. Possible role of prostacyclin synthase in altered prostacyclin generation in DOCA-salt hypertensive rats. Am J Hypertens 1991; 4: 667–673.
15. Baldwin, A.L, L.M. Wilson. Endothelium increases medial hydraulic conductance of aorta, possibly by release of EDRF. Am J Physiol 1993; 264: H26–H32.
16. Rodrigo GC, Denniff M. Time-of-day variation in vascular function. Exp Physiol 2016;101 (8): 1030–1034.
17. Uematsu, M., Y. Ohara, J.P. Navas, K. Nishida, T.J. Murphy, R.W. Alexander, R.M. Nerem, D.G. Harrison. Regulation of endothelial cells nitric oxide synthase mRNA expression by shear stress. Am J Physiol 1995; 269: C1371-1378.
18. Ranjan, V, Z. Xiao, S.L. Diamond. Constitutive NOS expression in cultured endothelial cells is elevated by fluid shear stress. Am J Physiol 1995; 269: H550-H555.
19. Pohl, U, K. Herlan, A. Haung, E. Bassenge. EDRF-mediated shear-induced dilatation opposes myogenic vasoconstriction in small rabbit arteries. Am J Physiol 1991; 261: H2016-2023.
20. Patrick, J, M.D. Casey, B. Jeffery, M.D Dattilo, M.S. Guohao Dai, A.James, B.S. Albert, I. Olga, M.D. Tsukurov, W.Roslyn, P. Jonathan, M.D. Gertler, M.W. Abbott,. The effect of combined arterial hemodynamics on saphenous venous endothelial nitric oxide production. J Vasc Surg 2001; 33:1199-1205.
21. Rubanyi, G.M. Endothelium-dependent pressure-induced contraction of isolated canine carotid arteries. Am J Physiol 1988; 255: H783-H788.
22. Hutcheson, I.R., T.M. Griffith. Release of endothelium-derived relaxing factor is modulated both by frequency and amplitude of pulsatile flow. Am J Physiol 1991; 261: H257-H262.
23. Kanai AJ, Strauss HC, Truskey GA, Crews AL, Grunfeld S, Malinski T. Shear stress induces ATP-independent transient nitric oxide release from vascular endothelial cells, measured directly with a porphyrinic microsensor. Circ Res 1995; 77: 284–293.
24. Laurindo, F.R.M., M.A. Pedro, H.V. Barbeiro, F. Pileggi, C. Carvalho, O. Augusto, P.L. de Luz. Vascular free radical release: ex vivo and in vivo evidence for a flow-dependent endothelial mechanism. Circ Res 1994; 74: 700–709.
25. Recchia, F.A., H. Senzaki, A. Saeki, B.J. Byrne, D.A. Kass DA. Pulse pressure-related changes in coronary flow in vivo are modulated by nitric oxide and adenosine. Circ Res 1996; 79: 849-856.

Corresponding Author

Dr Nidhi Priya, Assistant Professor, Department of Pathology, RUHS College of Medical Sciences, Jaipur, Rajasthan, India.

email: drnidhipriya@gmail.com