COMBINED EFFECTS OF PULSATILE SHEAR STRESS AND PRESSURE DRIVEN CYCLIC STRAIN ON PROTEIN EXPRESSION BY THE ISOLATED ENDOTHELIUM

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INTRODUCTION

The interior of the arterial system is lined with an active monolayer of cells, the endothelium, that provide a nonthrombogenic interface between the vessel wall and circulating blood elements. Endothelial cells respond to mechanical stimuli biochemically, through synthesis and expression of vasoactive proteins, growth factors, and thromboregulatory elements [1]. Endothelin-1 (ET-1) is a highly potent vasoconstrictor that binds to receptors on vascular smooth muscle cells. There it also mediates mitogenesis and could be responsible for the pathophysiologic smooth muscle cell proliferation observed in atherosclerosis [2]. Prostacyclin (PGI_2) is a vasodilator and strong inhibitor of platelet aggregation, which is a distinguishing characteristic of progressed atherosclerotic lesions [3]. Correlative studies have shown that regions of the vasculature associated with atherosclerosis have characteristic flow induced properties, specifically low mean and/or oscillating wall shear stress patterns [4-6]. In vivo, endothelial cells are subjected to several mechanical influences; flow-induced shear stress, pressure, and pressure-induced cyclic circumferential strain. Most previous investigations of endothelial responses to mechanical stresses have deconstructed the in vivo mechanical environment and only included the influence of either shear stress or cyclic stretch [7]. Zhao et. al. demonstrated that the combined effects of shear stress and cyclic strain on cell morphology are synergistic [8]. Moreover by defining the stress phase angle (SPA) as the temporal phase angle between pulsatile flow induced wall shear stress (WSS) and pressure pulse induced cyclic circumferential strain (CS), Qiu and Tarbell determined that the interaction effects between WSS and CS were statistically significant in the mediation of endothelial protein production [9]. These results illustrate the importance of considering the complete mechanical environment in endothelial studies. The aims of this investigation were to isolate the endothelium, reconstruct the complete in vivo mechanical environment, and evaluate endothelial biochemical responses to mechanical factors through the quantification of endothelin-1 and prostacyclin, proteins with properties that significantly influence the development of atherosclerosis.

METHODS

Porcine aortic endothelial cells were cultured on a compliant mock artery and subjected to pulsatile flow-induced shear stress and pressure pulse driven cyclic circumferential strain using a modified version of the flow system described by Moore et. al. [10]. This design allows precise and independent control of all flow characteristics (temperature, pressure, mean shear and shear amplitude, and circumferential strain). For the 48-hour experiments, shear and stretch parameters were chosen to model the mechanical conditions observed in both disease-prone, and immune regions of the vascular tree. Shear and stretch parameters were tested in multiple combinations. Considering time (0, 24, and 48 hours), shear stress ($\tau < 1$ dyne/cm^2, τ = 2+/-10 dynes/cm^2, and τ = 10+/-5 dynes/cm^2), and circumferential strain (0%, 3%, 6%, and 9%) as independent variables, the influence of each on protein expression as measured by radioimmunoassay, was determined by a repeated-measures ANOVA (SPSS 10.0, Chicago).

RESULTS

Based on the estimated marginal means, endothelin-1 production was inhibited by high pulsatile shear stress (10 +/- 5 dynes/cm^2) and unaffected by variance in cyclic strain (Figure 1). Differences in production related to low steady shear and oscillating shear were not significant through 48 hours. Prostacyclin production increased through 24 hours in all cases; then declined to basal levels from 24 to 48 hours in the absence of cyclic strain (Figure 2). Stretch ratios greater than 6% resulted in elevated prostacyclin concentration through 24 hours. By 48 hours, lower stretch ratios (3%) had also produced significant elevation of in the level of concentration. Varying shear stress did not significantly affect prostacyclin expression. Shear-stretch interaction effects were tested in both assays and did not produce statistically significant differences in protein expression at the time points observed in this study.

DISCUSSION

Elevated endothelin levels observed in experiments incorporating low mean and oscillating shear stresses reconcile well with correlative

studies linking these conditions to atherosclerosis and therefore could be responsible for the smooth muscle cell proliferation observed in these regions. Inhibition of prostacyclin in sites of low cyclic strain could be a factor promoting the platelet aggregation observed in advanced atherosclerosis as vessel compliance decreases with disease development. The importance of these results is that they were obtained using realistic reconstructions of the in vivo mechanical environment, combining physiologic levels of pulsatile flow-induced shear stress and pressure pulse driven cyclic circumferential strain, and are therefore more likely to represent the complex cellular biochemical activity that occurs in vivo.

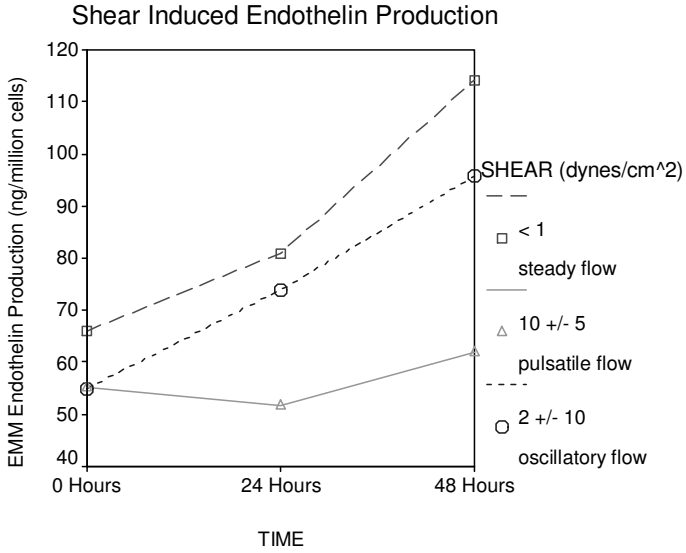

Figure 1. Comparison of the Estimated Marginal Means (EMM) calculated for endothelin production as a function of shear stress.

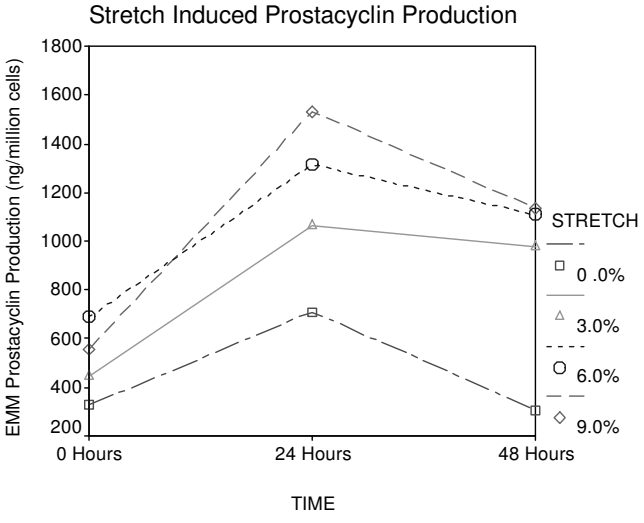

Figure 2. Comparison of the Estimated Marginal Means (EMM) calculated for prostacyclin production as a function of cyclic circumferential stretch.

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