MICROGRAVITY EFFECTS ON TRANSENDOTHELIAL TRANSPORT

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ABSTRACT

The endothelial cell (EC) layer which lines blood vessels from the aorta to the capillaries provides the principal barrier to transport of water and solutes between blood and underlying tissue. Endothelial cells are continuously exposed to the mechanical shearing force (shear stress) and normal force (pressure) imposed by flowing blood on their surface, and they are adapted to this mechanical environment. When the cardiovascular system is exposed to microgravity, the mechanical environmental of endothelial cells is perturbed drastically and the transport properties of EC layers are altered in response. We have shown recently that step changes in shear stress have an acute effect on transport properties of EC layers in a cell culture model, and several recent studies in different vessels of live animals have confirmed the shear-dependent transport properties of the endothelium.

We hypothesize that alterations in mechanical forces induced by microgravity and their resultant influence on transendothelial transport of water and solutes are, in large measure, responsible for the characteristic cephalad fluid shift observed in humans experiencing microgravity. To study the effects of altered mechanical forces on transendothelial transport and to test pharmacologic agents as counter measures to microgravity induced fluid shifts we have proposed ground-based studies using well defined cell culture models.

In future experiments, we will determine how alterations in hemodynamic forces (pressure and shear stress) affect transendothelial water and protein fluxes using endothelial cells cultured on porous filters and exposed to controlled pressure and shear forces in a unique rotating disk device. The role of intracellular biochemical signalling pathways which are known to be sensitive to mechanical forces will be probed with chemical inhibitors, and pharmacologic agents, known to affect these biochemical pathways, will be tested to determine if they can counteract the effects of altered mechanical forces on transendothelial transport properties.

INTRODUCTION

Blood vessel walls are lined by a monolayer of endothelial cells which, in addition to providing a blood contacting surface resistant to clotting, forms the principal barrier to transport of water and solutes across the wall. Regulation of water and solute transport is critical for controlling the chemical milieu of tissues serviced by individual vessels. Acute alteration of the endothelial transport barrier in response to the release of chemical agents (e.g., histamine, thrombin) produces local edema which is a central process in the inflammatory response (Ref. 1). Variations in endothelial resistance to transport of macromolecules such as low density lipoprotein are believed by many to play a role in the localization of atherosclerotic plaques in the arterial system (Ref. 2). Changes in transvascular pressure gradient associated with exposure to microgravity conditions in space travel lead to major fluid shifts between vascular and tissue compartments separated by endothelial barriers (Ref. 3). These fluid shifts affect physiological adaptation to space in profound ways (Ref. 4).

Redistribution of fluid volume and solutes between plasma and tissue spaces is dictated by complex physiological interactions among transport mechanisms operative at the level of the microvasculature. The basis for understanding fluid transport across the capillary wall is Starling's law

$$J_v = L_p A \left[\left(P_p - P_t\right) - \sigma\left(\pi_p - \pi_t\right) \right] \quad (1)$$

where J_v is the volumetric flow rate of fluid across the capillary wall, A is the surface area of the capillary, L_p is the hydraulic conductivity, P is the hydrostatic pressure, π is the oncotic pressure, and the subscripts p and t denote plasma and tissue, respectively. L_p and σ are properties of the capillary wall (endothelial cell layer), and the differences in hydrostatic pressure and oncotic pressure across the wall provide the driving forces for fluid transport. The oncotic pressure depends on the concentration of protein whose transport is governed by the equation

$$J_s = P_e A\left(C_p - C_t\right) + (1-\sigma)\, J_v \bar{C} \qquad (2)$$

where J_s is the solute (protein) flow rate, C is the solute concentration, $\bar{C}$ is the average concentration across the capillary wall, and P_e is the endothelial permeability, another transport property of the endothelial layer. Under normal conditions there is a slight imbalance between the hydrostatic and oncotic pressure driving forces in eqn. (1) leading to a net filtration from the plasma to the tissue at the arterial end of the microcirculation and a net resorbtion from the tissue to the plasma at the venous end. Any excess of filtration over resorption is normally compensated for by lymphatic flow which returns fluid to the venous system. It is conventional to think of membrane transport properties (L_p,σ,P_e) as constants, with changes in pressure and concentration gradients driving changes in water and solute flux. However, a fascinating characteristic of endothelial membranes which has become increasingly evident in the last few years is that their transport properties depend on the chemical and mechanical environment in which they reside.

Microgravity Induced Perturbations to the Mechanical Environment – When the body is exposed to microgravity, the blood pressure is reduced below the heart and elevated above the heart due to removal of the gravitational pressure gradient. As a result, the transvascular pressure gradient (P_p-P_t) and proportionate interstitial fluid flow (see eqn. 1) are reduced in the lower body (below the heart) and elevated in the upper body (above the heart). The net result is a resorbtion of fluid from the tissue space in the lower body and a filtration of fluid into the tissue space in the upper body. This leads to an overall shift of interstitial fluid from the lower to the upper body which manifests itself, for example, in the reduction in calf volume (resorbtion) and increase in facial volume (filtration) which are characteristically observed in astronauts exposed to microgravity (Ref. 4 and Ref. 5).

In addition to the significant changes in pressure forces on the endothelial cell lining of blood vessels which are induced by microgravity, there are changes in the tangential stress (shear stress) of flowing blood on the endothelial cell surface as well. The shear stress on the blood vessel wall (wall shear stress) is proportional to the blood flow rate, and it is well known that blood flow rate is perturbed in microgravity (Ref. 3). The flow perturbations associated with microgravity have been simulated by ground-based experiments involving elevation of limbs (Ref. 5) and whole-body tilt (Ref. 4 and Ref. 6). For example, forearm elevation, which reduces transmural pressure ((P_p-P_t) in eqn. 1), simulating exposure to microgravity in the lower body, leads to a reduction in blood flow (and associated wall shear stress) by a mechanism which is believed to involve post capillary vessel collapse producing increased flow resistance (Ref. 5). Similar trends in blood flow change have been observed in the lower extremities of human subjects exposed to -6° head-down-tilt (Ref. 4). Aratow et al. (Ref. 6) have observed that forehead cutaneous flow increases after exposure to -6° head-down-tilt.

In summary, the mechanical environment of endothelial cells exposed to microgravity can be characterized by: (1) a reduction in pressure and a reduction in shear stress for vessels in the lower extremities, and (2) an increase in pressure and in increase in shear stress for vessels in the head. These two types of mechanical perturbations will be imposed on our endothelial cell culture model and the responses of the membrane transport properties (L_p and P_e) will be determined (see question #1 below).

Effect of Flow (Shear Stress) on Endothelial Transport – The first unambiguous demonstration of a direct effect of shear stress on endothelial transport was reported by our group (Ref. 7) using bovine aortic endothelial cells (BAECs) cultured on a porous substrate mounted on the wall of a parallel plate flow chamber. This work showed a 10-fold increase in albumin permeability (P_e) within one hour after the onset of 10 dynes/cm^2 steady shear stress. The permeability returned to pre-shear baseline levels within two hours after the removal of shear stress. The absence of a boundary layer diffusion resistance was clearly demonstrated. More recent work by our group (Ref. 8) has shown a direct effect of shear stress on the hydraulic conductivity (L_p) of BAEC monolayers in culture. A significant increase in L_p was observed after 1 hour of exposure to 20 dynes/cm^2 of shear stress which did not return to baseline within 2 hours after removal of shear. This suggests that shear stress affects water and albumin transport across the endothelium in different ways.

Recent ex vivo and in vivo studies (refs. 9-14) provide mounting evidence that endothelial permeability increases as flow (wall shear stress) increases, as we have demonstrated in our in vitro system. To the best of our knowledge, there have not been any studies of the effects of changes in transmural pressure (P_p-P_t) on the transport properties (L_p, P_e) of endothelial layers. The experiments proposed under question #1 will be the first to examine this important question.

Cellular Control of Permeability – Macromolecular permeability of the endothelium is affected by many chemical mediators which activate second messenger systems within the cell. Histamine, bradykinin, and thrombin increase permeability of cultured endothelial monolayers by a mechanism of cell retraction involving cytoskeletal rearrangement of actin and myosin (Ref. 15). Cell retraction opens up the paracellular pathway between adjacent endothelial cells. Histamine, bradykinin, and thrombin receptors are coupled to guanine-binding proteins (G-proteins) which, when activated, increase the activity of phospholipase C (PLC) (Ref. 16). PLC cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) to yield two products, sn-1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). Both of these products act as second messengers. The second messenger role of IP_3 is to stimulate calcium release from its intracellular stores in the endoplasmic reticulum. On the other hand, DAG along with calcium and phosphatidylserine increase the activity of membrane bound protein kinase C (PKC). PKC phosphorylates specific serine and threonine residues in target proteins and causes an increase in endothelial permeability (Ref. 17). Intracellular calcium can also activate the calmodulin-dependent enzyme myosin light-chain kinase (MLCK), and the subsequent phosphorylation of myosin leads to cell retraction in endothelial cells (Ref. 15). These two pathways may interact since it is known that PKC can increase the sensitivity of contractile proteins to calcium (Ref. 18). Thus in cultured cells, an important mechanism for increased endothelial permeability is associated with a PLC mediated increase in intracellular calcium which via PKC and calcium-calmodulin induces contraction of cytoskeletal elements.

On the other hand, cellular relaxation associated with increased cell to cell contact has been proposed to decrease endothelial permeability (Ref. 19). Chronically elevated intracellular cAMP, maintained by a stable cAMP analogue, dibutyrl cAMP (db cAMP), decreases endothelial monolayer permeability to small molecules ranging in size from water to polyethylene glycol (Ref. 20). A similar reduction of sucrose transport results from stimulation of adenylate cyclase which elevates cAMP (Ref. 17). Intracellular pulses of cAMP have been shown to increase cell-cell contact, cell surface area and cell relaxation (Ref. 21). It has also been suggested that cAMP may modulate the activity of enzymes within the calcium-calmodulin-regulated actin-myosin contractile apparatus (Ref. 22).

Oliver (Ref. 23) showed that the sucrose permeability of BAECs in culture is reduced by endothelium-derived relaxing factor (EDRF) through a mechanism involving cGMP and suggested that this occurs as a result of cell relaxation which narrows the width of interendothelial junctions. On the other hand, Meyer and Huxley (Ref. 24) observed an increase in venular and true capillary L_p in the rat mesentery after perfusion with the nitric oxide (NO) donor sodium nitroprusside (SNP) or atrial natriuretic peptide (ANP), which is believed to be a NO-independent activator of cGMP. They did not observe a response of L_p to SNP or ANP in arteriolar capillaries. Kubes (Ref. 25) also observed an increase in

microvascular permeability after administration of ANP in the feline small intestine but surprisingly found that ANP exposure did not lead to an increase in tissue cGMP. Yet they still observed a reduction in permeability when cGMP was increased by other agents. Thus, the effects of NO and cGMP on barrier properties of the endothelium are somewhat controversial and appear to depend on the specific vascular origin of the endothelium.

From the studies outlined above it appears plausible that endothelial permeability is controlled by two transduction systems which modulate the transport resistance of the paracellular pathway. First, there is a mechanism involving increased intracellular calcium and PKC which increases permeability. Second, there is a mechanism involving increases in the cyclic nucleotides, cAMP and possibly cGMP, which decreases permeability. The relative activity of each of these systems may be responsible for overall control of endothelial permeability.

Biochemical Mechanisms of Shear Stress on EC Transport – In recent studies (Ref. 8) we have shown that preincubation of endothelial monolayers with db cAMP or the general phosphodiesterase inhibitor, IBMX, at levels which did not alter baseline L_p, completely inhibited (IBMX) or highly attenuated (db cAMP) the long-time increase in L_p observed with 20 dynes/cm^2 shear stress. The short-time decrease in L_p was completely blocked by these chemical agents as well. This indicates that elevation of the intracellular second messenger, cAMP, can oppose shear induced changes in L_p. We have also shown (unpublished) that the long-time shear-induced increase in L_p can be almost completely inhibited by preincubating the monolayer with the general G-protein inhibitor, GDP-βS. These results indicate that shear stress alters endothelial L_p by a cellular mechanism involving signal transduction.

EXPERIMENTAL DESIGN

In the microgravity fluid physics grant recently funded by NASA we will use in vitro cell culture models to explore the effects of altered hemodynamic forces associated with microgravity on the transport properties of vascular endothelium. The three major research questions and an overview of the associated methods are described briefly below.

Question #1: How do alterations in hemodynamics (pressure and wall shear stress) simulating microgravity affect endothelial transport properties (L_p and P_e)?

Overview: L_p and P_e of monolayers of representative arterial endothelial cells (bovine aortic endothelial cells – BAECs) and microvascular endothelial cells (bovine retinal microvascular endothelial cells – RMECs) will be measured simultaneously in a unique shearing apparatus (Ref. 8). The dynamic response of L_p and P_e to changes in transmural pressure and wall shear stress simulating exposure to microgravity hemodynamics will be determined over a time period long enough to achieve a steady state response (up to 6 hours).

Hemodynamic Conditions: Experiments will be run under "normal gravity" conditions to establish baseline values of L_p and P_e initially. It is anticipated that 1-2 hours will be required to establish steady state baseline values after initiating an experiment. Then step changes in transmural pressure (ΔP) and wall shear stress (τ_w) to a microgravity condition will be imposed, and L_p and P_e will be determined continuously for up to 5 additional hours or until new steady state values are obtained. Simulations of microgravity will initially involve step changes in transmural pressure of 20 cmH_2O and step changes in wall shear stress corresponding to 3-fold changes in flow rate. The magnitudes of these step changes can be varied. Alterations of the hemodynamics in 4 different circulatory regions will be simulated as indicated in Table I.

Questions #2: What biochemical pathways mediate the influence of pressure and shear stress on endothelial transport properties (L_p and P_e)?

Overview: The mechanism by which endothelial cells sense mechanical forces imposed by flowing fluids and transduce them to intracellular chemical signals are not clearly understood. Certain evidence (e.g., Ref. 26) indicates that it involves the activation of several classes of G-proteins while other evidence (e.g., Ref. 27) points to a role for surface integrin molecules as the transducing element.

Shear stress could activate G-proteins by transiently mimicking agonist binding or could mechanically perturb G-proteins directly. G-proteins, in turn, activate a myriad of signal transduction pathways to elicit physiological responses. Integrins can transmit surface shear forces to the cytoskeleton directly. There are at least two hypotheses to explain how endothelial cells control their transport properties. First, there is a mechanism involving increased intracellular calcium, MLCK, and PKC that leads to cytoskeletal rearrangements, resulting in cell retraction of endothelial cells to increase P_e and L_p. A second hypothesis suggests that cyclic nucleotides, cAMP and possibly cGMP increase endothelial barrier function by enhancing cell to cell contact, resulting in decreased endothelial transport properties. The objective of this part of the study is to elucidate the roles played by these signal transduction pathways in pressure and shear-induced alterations in P_e and L_p. By utilizing various inhibitors and modulators of the signal transduction components under defined hemodynamic conditions while simultaneously measuring L_p and P_e, we will test these two hypotheses.

Question #3: Can the transport properties of endothelial monolayers be manipulated by pharmacologic agents in the hemodynamic environment of microgravity?

To reduce net resorbtion of fluid from tissue space in the lower extremities under microgravity conditions it would be desirable to increase L_p on the arterial side to enhance filtration and to reduce L_p on the venous side to inhibit resorbtion. To reduce net filtration in regions above the heart it would be useful to reduce L_p on the arterial side and increase L_p on the venous side. In this phase of the proposed research we will test pharmacologic agents in order to identify those which are capable of significantly enhancing or inhibiting the transport properties of the endothelium under the hemodynamic conditions of microgravity. The search for effective drugs will be guided by our knowledge of the biochemical pathways which control endothelial transport as determined under question #2. We realize at the outset that there are potential problems associated with the use of drugs to provide counter measures to microgravity induced physiological changes. Successful drugs need to be as site specific as possible with minimal systemic side effects. It may, however, ultimately be possible to use modern drug delivery technologies to obtain local delivery with minimal systemic effects.

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