THE EFFECT OF INDOMETHACIN ON HEALTHY FROG CAPILLARIES FOLLOWING A SHEAR STRESS STIMULUS: POTENTIAL CLINICAL IMPLICATIONS

by

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Carmen Kay Staigmiller

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ABSTRACT

When cultured endothelial cells are stimulated by shear stress they release prostaglandins. One of the effects of prostaglandin release is vasodilation. The effect of prostaglandins on hydraulic conductivity (Lp) is not known. Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that is known to block prostaglandins. The purpose of this study was to evaluate the effect of indomethacin on endothelial cells of a true capillary. It was hypothesized that in an intact healthy capillary, superfused with indomethacin, there will be a decrease in capillary Lp after a shear stress stimulus. The mesentery of healthy North American leopard frogs (n=16) was exposed and a capillary was cannulated. Using the modified Landis technique Lp was assessed, after a shear stress stimulus, in a control capillary and a capillary that was exposed to indomethacin. There was not a significant decrease (P=0.13) in Lp when comparing the control vessels with treatment vessels. Data from this study suggest that prostaglandins are not involved with the response of Lp to shear stress in healthy frogs. Since Lp did not decrease in the presence of indomethacin, it is probable that other processes are affecting the response of the endothelium. The clinical implications of this study are important because of the potentially devastating impact of the toxic effects of NSAIDs.

CHAPTER 1

INTRODUCTION

A family nurse practitioner (FNP) working in the emergency department (ED) of a Critical Access Hospital in rural Montana admits a thirty year old male presenting with signs and symptoms of sepsis. He is delirious, tachycardic, diaphoretic, febrile, and acidodic. The patient's blood pressure is 70/30 after three liters of fluid, and as a result a vaso-pressor is started. As the nurse practitioner cares for the patient, she realizes the patient has "third spaced" most of his fluids. Questions formulate in her mind, "Where did all the intravenous fluids go? What happened in the vasculature that allowed this fluid to escape out of the vessels? What intervention will bring the fluids back into the vasculature so blood pressure and perfusion are maintained?"

"Third spacing" or capillary leak is the movement of fluid from the vasculature into the interstitial tissue as a result of endothelial dysfunction (Aneja & Carcillo, 2009). This phenomenon can be found in patients with multiple disease processes such as renal failure, heart failure, liver disease, sepsis, systemic inflammatory responses and trauma (Aneja & Carcillo, 2009). Body fluid, or plasma, is a medium in which gases, water, nutrients, blood cells, minerals, vitamins, antibodies, and hormones are carried to the cells (Thomas, 1993). Plasma also removes waste, formed during metabolism, away from the cell structure (Thomas, 1993). There are two general types of fluid reservoirs in the body: intracellular and extracellular. Extracellular is further divided into interstitial and

extravascular. Fluid moves freely between these spaces influenced by capillary permeability, hydrostatic pressure and oncotic pressures (Rose B. D., 2009).

Body fluids should be in balance with the amount of fluid entering the body equaling the amount exiting the body via perspiration, urine, stool, and respiratory effort. Edema, defined as an expansion of the interstitial fluid volume, is a result of an alteration in capillary hemodynamics that results in the movement of fluid from the vascular space into the interstitium (Rose B. D., 2009). The movement of fluid from the plasma into the interstitium is represented by Starling's law: net filtration = LpS x (Δ hydrostatic pressure – Δ oncotic pressure) (Rose B. D., 2009). Lp being permeability of the capillary and S is the surface area that the fluid is moving through (Rose B. D., 2009).

In the presence of severe systemic infection, a sepsis syndrome may develop. Sepsis is characterized by inflammation, vasodilation, increased microvascular permeability and leukocyte accumulation (Neviere, 2009). These processes often result in third spacing of fluid which results in hypotension and decreased tissue perfusion (Rose B. D., 2009). There is increasing evidence that changes in the endothelium are important determinants of the pathophysiology of sepsis (Aird, 2009).

A FNP is the primary care provider at a rural clinic in Montana. The nurse practitioner has been caring for a diabetic patient for numerous years. Over the course of time, the patient begins to develop signs of microvascular disease including microangiopathy, nephropathy, neuropathy, and changes indicating retinopathy on dilated eye examination. The nurse practitioner knows that clinical evidence shows tight management of blood glucose can delay the vascular effects of diabetes; however, despite

the best diabetes management and a motivated patient, changes in the microvasculature eventually occur (Cotran, Kumar, & Collins, 1999).

Perrin, Harper, & Bates, (2007) demonstrated that diabetes mellitus (DM) causes an increased permeability or increased hydraulic conductivity (Lp) in the vascular endothelium which is believed to result in the angiopathy that is part of the diabetic milieu. However, this mechanism is poorly understood. The complications associated with diabetes are believed to be a result of metabolic aberrancy, commonly elevated glucose (Cotran, Kumar, & Collins, 1999). The increase in glucose and glucose metabolites result in impaired ion pumps and increased intracellular osmolarity resulting in an influx of water (Cotran, Kumar, & Collins, 1999). What can FNP's do to prevent these debilitating effects of disease?

A fifty year old man arrives in a Critical Access hospital ED presenting with chest pain, nausea, diaphoresis, and hypertension. His electrocardiogram is positive for ischemic changes and he is air lifted to the nearest medical facility with a cardiac catheterization lab. During the cardiac arteriogram, a 90% occlusion of his left anterior descending artery and 70% occlusion of the circumflex artery were noted. Why has this patient developed such diffuse coronary heart disease? Often myocardial ischemia is the result of atherosclerosis, or the buildup or abrupt change of plaque causing narrowing of the arteries which results in diminished blood flow (Cotran, Kumar, & Collins, 1999). "Coronary endothelial dysfunction is a common finding in patients with coronary artery disease" (Aird, 2009, p. 10). "In patients with coronary heart disease, an elevated serum

level of C-reactive protein, a marker of chronic inflammation, is an independent predictor of endothelial dysfunction" (Mohler, 2009, p. 3).

Donna Williams PhD, a bench scientist studying microcirculation and control of capillary hydraulic permeability in intact, living capillaries, conducts research on the physiology of capillary hydraulic conductivity in the endothelium of true capillaries in the North American leopard frog. Dr. Williams' goal is to compare the results of her research, to the current understanding of human physiology, and subsequently improve our understanding of the function of capillaries and endothelial cells and the role they play in health and disease. Increased understanding of microcirculation may lead to improved disease and trauma management of humans.

Working with Dr. Williams in this study provided an opportunity for this author to gain an increased understanding of the research processes, human physiology, and its application to clinical practice. As a future primary healthcare provider, utilizing the results of bench science will provide a strong scientific base for practice decisions. The author's goal is to encourage nurse practitioners to develop a greater understanding of physiology and apply this understanding into their everyday practice. Participating in this study brought to the forefront the importance of understanding the function of capillaries and endothelial cells in response to disease processes. If nurse practitioners understand the physiological process at the level of the endothelial cell, it will allow the nurse to make more informed clinical decisions about interventions, medications, and patient care. Nurse practitioners are encouraged to collaborate with bench scientists to broaden the vision of both disciplines and improve patient outcomes.

Nurse practitioners provide a holistic approach to primary care which includes not only science but also the art of nursing that facilitates to connections with others. The art of nursing is intimately involved in personal care of patients which includes bathing, feeding, toileting, and dressing. In addition, many patients feel comfortable enough to reveal their inner most secrets. Nurse practitioners are unique in their ability to blend science and human caring to facilitate the best possible health outcomes.

Purpose

Endothelium is a monolayer of endothelial cells that line the complete vasculature (Cotran, Kumar, & Collins, 1999). "Their structural and functional integrity is fundamental to the maintenance of vessel wall homeostasis and circulatory function" (Cotran, Kumar, & Collins, 1999, p. 496). The multiple functions of the capillaries and endothelium can be stimulated by various changes in its environment. Stimulation of the endothelium can result in a rapid response or occur over hours to days (Cotran, Kumar, & Collins, 1999). Activation or stimulation of the capillary endothelium and its response is coming to the forefront of research because of the implications for disease regulation (Cotran, Kumar, & Collins, 1999). The purpose of this study was to investigate the effects of indomethacin on endothelial cells of an intact true capillary.

This research will contribute to science by increasing the understanding of how capillary endothelium functions thereby providing scientific evidence for future medical related interventions. A greater understanding of capillary endothelial physiology will enhance the provider's ability to understand the disease process and the effect of

interventions at the cellular level. This in turn enables the nurse practitioner to provide educated and evidence based interventions that will produce desired physiological responses. Appropriate evidence based interventions that are established using an understanding of cellular physiology will then result in prudent medical interventions for the patient.

Background and Significance of Study

Scientists once believed endothelial cells were "merely a passive barrier, lining the inner walls of blood vessels" (Kvietys & Granger, 1997, p. G1189). After years of research and inquisition the current state of the science of endothelial cells is "that vascular endothelium is an active metabolic component of tissues that serves a number of important physiological functions" (Kvietys & Granger, 1997 p G1189). Perhaps, one of the most significant advances in the study of capillary endothelial cells was developed in 1927 by E. M. Landis and subsequently modified by Michel (Michel, Mason, Curry, Tooke, & Hunter, 1974). Landis and later Michel, developed a technique that quantitatively measured "the rate of filtration and reabsorption of water across the capillary walls" (Michel, Mason, Curry, Tooke, & Hunter, 1974). This technique and many other scientific studies have contributed to the transformation of scientists or physiologists understanding of capillary endothelial cells.

Vascular endothelial cells are known to have the following physiologic functions: 1) regulation of microvascular fluid and solute exchange through cell contraction, 2) maintenance of antithrombogenic vessel surface through production of prostaglandin I_2

and nitric oxide (NO), 3) modulation of vessel proliferation through production of adenosine, 4) control of leukocyte trafficking through surface expression of adhesion molecules, 5) regulation of vascular tone and blood flow through production of NO, adenosine, endothelin, and other substances, and 6) metabolism of circulating molecules through surface expression of enzymes (Kvietys & Granger, 1997). It is also known "that abnormal endothelial cell responses frequently accompany organ dysfunction and disease" (Kvietys & Granger, 1997 p G1189). What is not known is the mechanism that triggers the abnormal response and how it is communicated within the capillary, endothelial cells, and surrounding tissues.

Statement of Problem

Dr. Williams' current research addresses the effect of indomethacin, a non-steroidal anti-inflammatory drug, on capillary endothelial hydraulic conductivity after a shear stress stimulus. Acquiring an understanding of these effects will contribute to the current state of the science of endothelial cells.

Hypotheses

In an intact healthy capillary, superfused with indomethacin, there will be a decrease in capillary hydraulic conductivity (Lp) after a shear stress stimulus.

Operational Definitions

Indomethacin: “Reversibly inhibits cyclooxygenase-1 and 2 (COX-1 and 2) enzymes, which result in decreased formation of prostaglandin precursors; has antipyretic, analgesic, and anti-inflammatory properties” (Lexi-Comp, 1979-2009, p.2)

Capillary hydraulic conductivity (Lp): Rate per unit pressure of water moving across the capillary wall.

Endothelial cells: an interactive organ system that forms the inner lining of all blood vessels; that “mediates vasomotor tone, regulates cellular and nutrient trafficking, maintains blood fluidity, contributes to the local balance between pro- and anti-inflammatory mediators as well as procoagulant and anticoagulant activity; participates in generation of new blood vessels, interacts with circulating blood cells, and undergoes programmed cell death” (Aird, 2009,p.1).

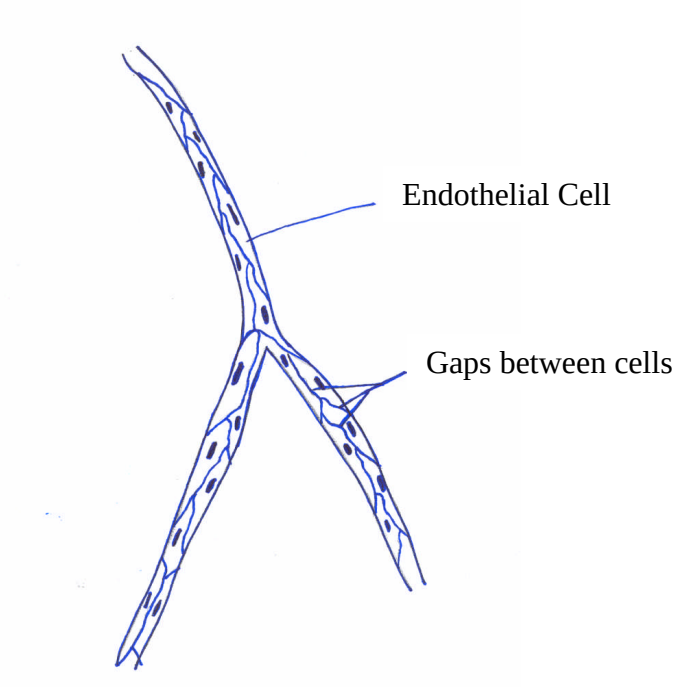


Figure 1. Individual Capillary Adapted from Gray (2000).

True capillary: branch mainly from the arteriole end of a blood vessel and merge with venules. They are lined with endothelial cells that have narrow, tight gaps that provide exchange between cells and the circulation. Many capillaries form from each arteriole resulting in a large total area creating a slow, steady blood flow (Cotran, Kumar, & Collins, 1999).

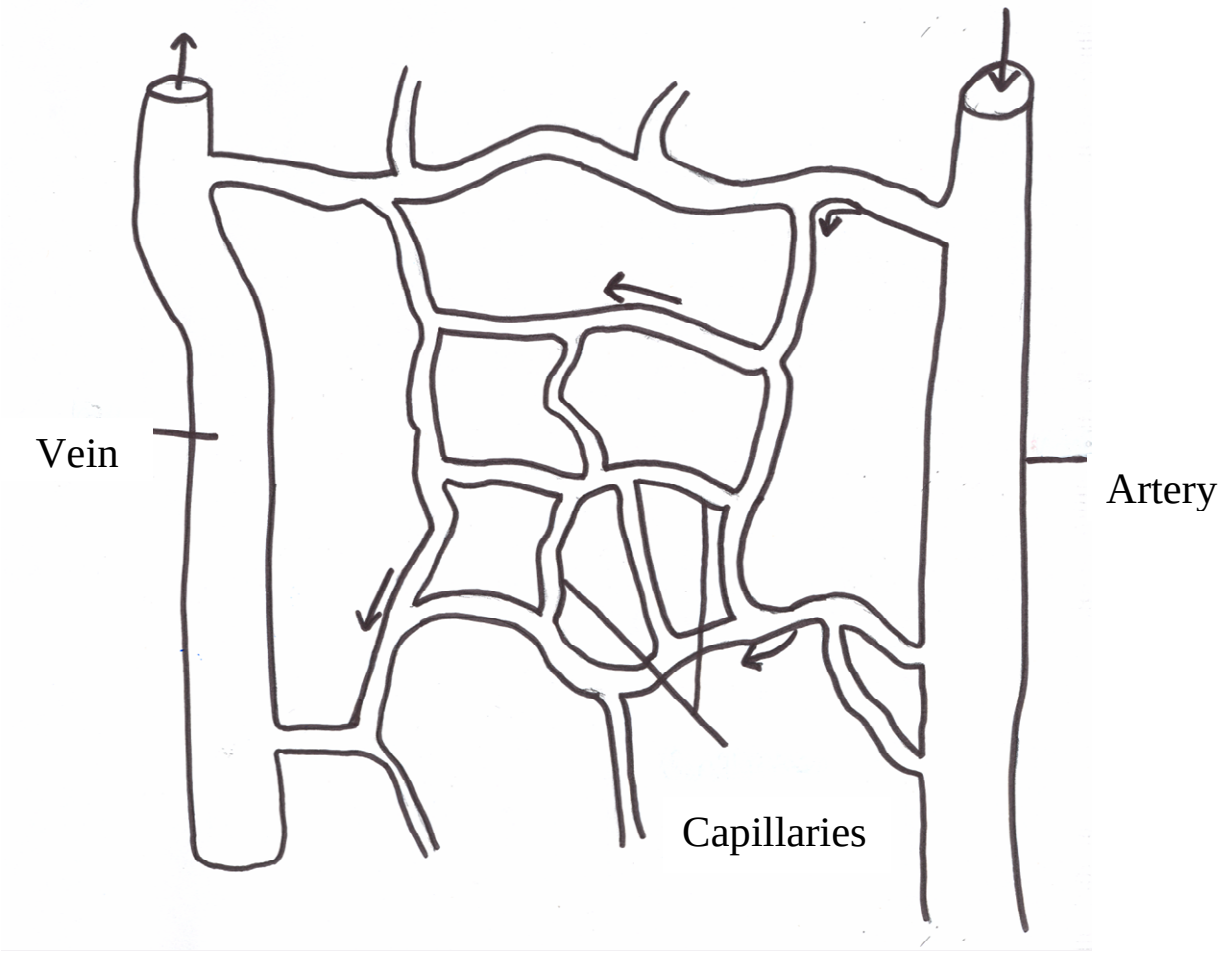


Figure 2. Capillary Bed, Adapted from Doohan (1999)

Shear stress: physical stimuli to endothelial cells, that is a frictional force acting in the direction of blood flow on the inner surface of blood vessels (Ueba, Kawakamii, & Yaginumm, 1997), (Mohler, 2009).

Bench science: hypothesis driven experimental science to advance knowledge.

Assumptions

1. It is assumed that hydrostatic pressure is the same across all regions of the vascular wall, this could lead to underestimating Lp and an over estimation of osmotic pressure (Clough, Michel, & Phillips, 1988).
2. The combination of a wild caught frog, a normal white blood cell count, and no rolling or sticking leucocytes seen when visualizing the capillary, resulted in the assumption that each frog was healthy and there was no inflammation.
3. Indomethacin was superfused over the mesentery and then was assumed to reach the capillary bed to exert its effects.
4. In calculating Lp it is assumed that the capillaries are cylinders and when a cross-section is done the capillary is circular (Michel, Mason, Curry, Tooke, & Hunter, 1974).
5. It is also assumed that pithing the frog does not alter the neurologic response of the capillary or change the flow to the capillaries.
6. The final assumption is that exteriorizing the mesentery does not affect blood flow to the capillary bed.

Limitations

The limitations of this study are related to the use of the North American leopard frog and not actual human tissue. This study cannot be generalized to other types of mesenteric endothelial cells such as humans. Studies that examined the similarities between human and frog mesentery microvessels found that there are “extensive structural similarities” (Bundgaard & Frokjaer-Jensen, 1982, p.1). This makes “frog mesenteric capillaries a useful model of mammalian continuous capillaries” (Bundgaard & Frokjaer-Jensen, 1982). A second limitation is that the experiment was conducted at 15 ˚C as this is normo-thermic for amphibians.

CHAPTER 2

REVIEW OF THE LITERATURE

A literature review was conducted to gain an understanding of the current state of knowledge of endothelial cells and the movement of fluid from capillaries. Articles in this literature review were found in the databases of CINAHL and Medline.
The primary themes covered in this review were shear stress, fluid movement, prostaglandins, and indomethacin.

The results of this literature review are summarized below beginning with shear stress. Shear stress is an increase in frictional force exerted on the endothelial cells causing stimulation of certain cell responses. Multiple studies were conducted using cultured endothelial cell monolayers where shear stress was used to stimulate the multiple reactions of the endothelium.

Shear Stress

Sill, Chang, Artman, Frangos, Hollis, & Tarbell, (1995) hypothesized that hydraulic conductivity (L_p) may be altered by shear stress and that these alterations may be cell mediated through signal transduction mechanisms and could be inhibited by pharmacological intervention. Sill, et al. (1995) concluded that shear stress altered endothelial L_p through a cellular mechanism involving signal transduction, not by a purely physical mechanism. Other studies investigated mechanotransduction of shear stress by the vascular endothelium. The authors imposed shear stress on cultured cells

and studied the induced changes in the membrane fluidity (Butler, Norwich, Weinbaum, & Chien, 2001) (Haidekker, L'Heureux, & Frangos, 2000). Cell membrane fluidity is a property that has been strongly associated with cell functions that affect cell membrane protein diffusion (Butler, Norwich, Weinbaum, & Chien, 2001). Many of the flow-induced responses are mediated by G protein activation; however, the primary mechanosensor that transduces the mechanical stimulus (shear stress) into a biochemical signal is unknown (Haidekker, L'Heureux, & Frangos, 2000). Haidekker et. al (2000), concluded that shear stress increased membrane fluidity of the endothelial cell and hypothesized that this change in fluidity resulted in a mechanochemical transduction across the cell membrane stimulating the many responses of the endothelial cells.

The effects of shear stress on endothelial cells have also been linked to the generation of atherosclerotic plaques in blood vessels (Haidekker, White, & Frangos, 2001). Haidekker, White, & Frangos, (2001) reported that sudden, disturbed flow changes resulted in stimulation of endothelial cells to proliferate, resulting in an increase in atherosclerotic plaques. Other studies attempted to understand the signal transduction mechanism that stimulated atherogenesis after an abrupt decrease in shear stress (Ziegelstein, Blank, Cheng, & Capogrossi, 1998) (Murase, et al., 1998). Ziegelstein, Blank, Cheng, & Capogrossi, (1998) concluded that an acute reduction in fluid shear stress activates an increase in cytosolic pH in the vascular endothelial cells. Murase, et al., (1998) observed that shear stress modulated endothelial functions including gene expression of LOX-1 which is a receptor site for atherogenic oxidized LDL.

Chang, Yaccino, Lakshminarayanan, Frangos, & Tarbell, (2000) studied the effect of nitric oxide (NO) on the increased hydraulic conductivity (Lp) of endothelial cells that was stimulated by an increase in shear stress. The authors reported that the proposed pathway of NO release was not the pathway that mediated the shear stress L_p response (Chang, Yaccino, Lakshminarayanan, Frangos, & Tarbell, 2000).

Multiple studies have been done investigating the shear stress stimulated response of endothelial cells to release prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2). Frangos, Eskin, McIntire, and Ives (1985) established that the production of PGI_2, by cultured vascular endothelium cells exposed to pulsatile shear stress, is significantly higher than previous studies that used stationary flows. These authors also believed that the lower production of PGI_2 in the veins, as compared to arteries, is due to less forceful pulsatile flow in the veins (Frangos, Eskin, McIntire, & Ives, 1985). Waters (1996) observed that blocking the production of NO and cyclooxygenase did not alter the increase in cultured endothelial cell permeability related to increased shear stress. Tsao, Lewis, Alpert, and Cooke (1995) investigated NO and prostacyclin release after a shear stress stimulus. The authors used chemicals to block the production of NO and prostacyclin and then monitored the adhesiveness of monocytes to the endothelial cells. These authors concluded that shear stress alters endothelial adhesiveness for monocytes and that this effect is largely due to the stimulation of NO with prostacyclin having a lesser effect (Tsao, Lewis, Alpert, & Cooke, 1995).

Capillary Filtration Coefficient

Capillary filtration coefficient (CFC) is defined as the change in transcapillary fluid exchange induced by a fixed change in transcapillary hydrostatic pressure (Bentzer, Kongstad, & Grande, 2001). CFC was established as another method to estimate capillary hydraulic conductivity (Kongstad & Grande, 1998). This method used a denervated cat skeletal muscle that continued to have vascular control and was perfused with autologous blood (Kongstad & Grande, 1998). The CFC was measured in the presence of multiple physiologic forces such as varied arterial pressure, vascular tone, blood flow, and number of perfused capillaries. It was concluded that CFC can be used to study capillary L_p (Kongstad & Grande, 1998) (Bentzer, Kongstad, & Grande, 2001). Following this conclusion, studies were conducted to evaluate the effects of various substances on CFC. Bentzer, Holbeck, and Grande (2002) concluded that CFC was decreased; therefore, capillary L_p was decreased, after a secondary release of prostacyclin. Persson, Ekelund, & Grande, (2003) evaluated exercise induced release of NO and prostacyclin and its effect on vascular permeability. The authors observed that microvascular hydraulic permeability was reduced during exercise, decreasing muscle edema and was regulated by the release of NO (Persson, Ekelund, & Grande, 2003).

Lundblad, Bentzer, & Grande, (2003) believed Rho kinases provide part of the regulation of the contractility of the cytoskeleton in endothelial and smooth muscle cells by influencing the size of the gaps between endothelial cells (Lundblad, Bentzer, & Grande, 2003). The CFC technique was used to measure L_p after varied doses of Rho kinase inhibitor. Lundblad, Bentzer, and Grande (2003) concluded that Rho kinase is

involved in regulation of microvascular permeability. A similar follow up study evaluated the effects of Rho kinase inhibition and cAMP stimulation on capillary permeability when endotoxemia or inflammation was present (Lundblad, Bentzer, & Grande, 2004). The authors reported that the increased permeability during endotoxemia was caused by other pathways than the ones affected by cAMP stimulation and Rho kinase inhibition (Lundblad, Bentzer, & Grande, 2004).

Landis Technique Without Shear Stress

Another method for measuring L_p was the development of the micro-occlusion technique or Landis technique (Clough, Michel, & Phillips, 1988). This technique used frog mesenteric capillaries because there are "extensive structural similarities between microvessels of the frog mesentery and those of mammalian skeletal muscles…, indicating that frog mesenteric capillaries are a useful model of mammalian continuous capillaries in general" (Bundgaard & Frokjaer-Jensen, 1982, p. 1). After cannulation of the capillary with a micropipette, a solution containing a small amount of human red blood cells was infused (Michel, Mason, Curry, Tooke, & Hunter 1974). The capillary was then occluded downstream, the movement of the red cells was believed to be as a result of fluid moving across the capillary wall (Michel, Mason, Curry, Tooke, & Hunter 1974).

Multiple studies have used modifications of the Landis technique combined with exposing the capillary bed to multiple variables. For example, Clough, Michel, & Phillips, (1988) exposed the capillary bed to increased temperatures. They observed that

exposure to temperatures of thirty to thirty-five degrees Celsius increased L_p in capillaries and venules (Clough, Michel, & Phillips, 1988). The authors also observed an increase in L_p in inflamed microvessels and believed this was associated with the development of gaps between endothelial cells and that increased permeability resulted in a deterioration in structural integrity of the vessel (Clough, Michel, & Phillips, 1988).

Blumberg, Clough, and Michel (1989) demonstrated that flavonoids decrease the effects of histamine and bradykinin which are known to increase Lp. Exposure of the endothelial cells to hydroxyethyl rutosides (HR), a flavonoid, decreased microvascular permeability (Blumberg, Clough, & Michel, 1989). Williams and Huxley (1993) exposed the endothelial cells to bradykinin, a vasodilator. These authors reported an increase in L_p, the increase in L_p was dependent on where the capillary was located in the microvascular network and at what time the measurement was taken.

Hargrave, Huxley, & Thipakorn (1995) investigated changes in L_p and overall volume status of the frog in correlation with the varied seasons. The authors reported that true capillary L_p was stable throughout the seasons but the overall volume status varied cyclically throughout the year. They concluded the lack of a relationship between L_p and volume status suggests an independent stimulus such as a hormonal signal for hydraulic conductivity (Hargrave, Huxley, & Thipakorn, 1995).

Victorino, Newton, & Curran (2003) and Victorino, Newton, & Curran (2004) reported endothelin-1 (ET-1) and Angiotensin II are potent vasoconstrictors that are released during shock and/or sepsis. Victorino, Newton, & Curran (2004) also reported that exposure of the endothelial cells to ET-1 attenuated the increase in microvascular

permeability. ET-1 could then be concluded to have vasopressor function and have the ability to decrease third-spacing of fluid. It is interesting to note that exposing endothelial cells to angiotensin II, also a vasoconstrictor, revealed a dose-dependent increase in microvascular permeability (Victorino, Newton, & Curran, 2004).

Growth factors such as vascular endothelial growth factor (VEGF) are often noted in a variety of conditions were angiogenesis and leaky vessels are present (Bates & Curry, 1996). Using the Landis technique and exposing the endothelial cells to VEGF resulted in an acute and chronic increase in L_p (Bates & Curry, 1996). A subsequent study by Pocock, Williams, Curry, & Bates (2000) investigated three different hypothesis of how VEGF increased Lp. The authors reported their data to be consistent with the theory that VEGF acts through a Ca^2- store-independent mechanism and ATP acts through Ca^{2+} store-mediated Ca^{2+} influx (Pocock, Williams, Curry, & Bates, 2000). Angiopoietin-1, also a growth factor, was reported to decrease L_p through modification of the endothelial glycocalyx (Salmon, Neal, Sage, Glass, Harper, & Bates, 2009).

Landis Technique and Shear Stress

The study of hydrostatic and oncotic pressures influence on the capillary bed started in the late 1800's by Starling (Michel, Mason, Curry, Tooke, & Hunter, 1974). It has been believed that hydrostatic pressure is the predominant force affecting the capillary bed (Williams, 1999). Since then studies on cultured endothelial cells indicated that shear stress also influenced the cell barrier functions. Subsequent studies have

investigated the effects of shear stress on intact capillaries (Williams, 1999; Williams, 2003; Kim, Harris, & Tarbell, 2005).

Williams (1999) reported that L_p increased proportionately to the increase in shear stress. It was also observed that true capillaries and low pressure venular capillaries were affected by changes in shear stress when arteriolar capillaries were not (Williams, 1999). Williams (1999) concluded that capillaries adjust filtration rate in response to shear stress. Williams (2003) reported venular capillaries responded to different magnitudes, rates, and patterns of changes in shear stress. Hydraulic conductivity of capillaries was affected by both the rate and the magnitude of changes in fluid shear stress (Williams, 2003). A similar study using autoperfused rather than cannulated capillaries also revealed an increase in shear stress resulted in an increased L_p (Kim, Harris, & Tarbell, 2005). Once the relationship between shear stress and L_p was discovered, Williams (2007) attempted to understand this relationship. The endothelial glycocalyx was disrupted with pronase, a protease enzyme used to target the glycocalyx of the capillary lumen, and the effects of shear stress on L_p were measured (Williams, 2007). The relationship between shear stress and L_p was maintained and strengthened after pronase treatment. These results were then interpreted as evidence that components of the capillary lumen decrease the effects of a shear stress stimulus (Williams, 2007).

Prostaglandins

The study of prostaglandins dates back to the 1930's when they were observed to have a stimulatory effect on smooth muscle tissues and were believed to be produced in

the prostate gland; therefore, named prostaglandin (Griffing & Witherow, 2008). Prostaglandins, prostacyclin (PGI_2), prostaglandin D_2 (PGD_2), prostaglandin $F_{2\text{-alpha}}$, and prostaglandin E_2 (PGE_2), are formed when cyclooxygenase (COX) metabolizes arachidonic acid (Griffing & Witherow, 2008). The microvascular endothelium primarily produced PGI_2 (Griffing & Witherow, 2008). PGI_2 is known to inhibit platelet aggregation, cause vasodilation, and reduce the aggregation of cholesterol and to reduce the generation of vascular smooth muscle cells (Schonbeck, Sukhova, Graber, Coulter, & Libby, 1999).

Platelet Aggregation

Prostacyclin in conjunction with NO has been studied in relation to platelet aggregation in vitro and in vivo. Broeders, Tangelder, Slaaf, Reneman, and oude Egbrink (2001) reported that PGI_2 and NO have a "synergistic effect" that protects an arteriole which has encountered wall injury from continued thromboembolism. This synergistic effect was also found to be protective against continued enlargement of thromboembolism in arterioles caused by shear stress (Broeders, Tangelder, Slaaf, Reneman, & oude Egbrink, 2001).

Chardigny, Van der Perre, Simonet, Descombes, Fabiani, and Verbeuren (2000) studied PGI_2 as a vasodilator and inhibitor of platelet aggregation in coronary artery bypass surgery. The authors concluded that PGI_2 was more effective in the internal mammary artery (IMA) then the radial artery, resulting in fewer post-op complications of

narrowing and occlusion when the IMA was used (Chardigny, Van der Perre, Simonet, Descombes, Fabiani, & Verbeuren, 2000).

Hypercoagulopathy, a result of endothelial cells dysfunction in the pulmonary arterioles, is believed to cause primary pulmonary hypertension (PPH) (Pnedman, Mears, & Barst, 1997). Patients with PPH were known to respond to treatment with PGI_2 but its mechanism was unknown. Pnedman, Mears, & Barst (1997) reported that the long term use of PGI_2 normalized the hypercoagulopathy state in the pulmonary arterioles resulting in a decrease in endothelial cell injury.

Vasodilation

Multiple studies, Messina, Sun, Koller, Wolin, & Kaley (1992), Kato, Iwama, Okumura, Hashimoto, Ito, & Satake (1990), Mortensen, Gonzalez-Alonso, Bune, Saltin, Pilegaard, & Hellsten (2009), and Engelke, Halliwill, Proctor, Dietz, & Joyner (1996) investigated the effect of prostaglandins on various types of tissues including, aorta and skeletal muscles from rats, and forearm and leg muscles from humans. All of these authors reported the release of prostaglandins from endothelial cells as a result of varied stimulus, elicited vasodilation and control of blood flow.

PGI_2 is a known vasodilator however; its effects vary depending on concentration and tissue type. In fact PGI_2 can produce vasoconstriction, rather than vasodilation, when produced in a higher concentration and on certain tissue types (Williams, Dorn II, & Rapoport, 1994). When these authors used rat aorta cells, they reported that PGI_2 induced dilation through a PGI_2-PGE_2 receptor. When PGI_2 is produced in higher concentrations

it acts on another receptor (thromboxane) resulting in a decrease in vasodilation effect (Williams, Dorn II, & Rapoport, 1994). Bagi, et al., (2005) reported that the endothelium of mice with type 2 diabetes mellitus (T2-DM) responded differently and had an increased release of constrictor prostaglandins resulting in an increased peripheral resistance and blood pressure.

Multiple studies investigated the effect of shear stress on vessels walls and the corresponding release of prostaglandins. Koller, Dornyei, and Kaley (1998) noted an increase in shear stress, stimulated an increased release of dilating prostaglandins and NO. The vasodilation resulting from the release of prostaglandins worked to control shear stress and vascular resistance. Sun, et al., (1999) reported an increased production of dilator prostaglandins from the endothelium in response to a shear stress stimulus in mice that genetically lacked the ability to produce NO. A following study used rats with pre-existing hypertension to study the alteration of shear stress-induced vasodilation from prostaglandin release (Huang, Sun, & Koller, 2000). The results revealed an increased release of prostaglandin H_2 (PGH_2) that caused a reduced shear stress-dependent dilation, which was believed to cause an increased vascular resistance in hypertension (Huang, Sun, & Koller, 2000). The release of prostaglandins as a result of shear stress has also been hypothesized to increase intestinal glucose uptake because of an increased blood flow (Han, Ming, & Lautt, 1999). It was reported by Han, Ming, & Lautt (1999) that glucose uptake was inhibited by indomethacin, a COX inhibitor, but glucose uptake was also stimulated by exposure to prostaglandin F_2 (PGF_2).

C-reactive protein (CRP) is a marker of inflammation and cardiovascular disease and is known to inhibit the actions of NO (Hein, Qamirani, Ren, & Kuo, 2009). Hein, Qamirani, Ren, & Kuo (2009) investigated the effect of CRP on the COX mediated production of prostanoids (PGI_2, PGE_2) and its vasomotor pathway. These authors determined that CRP "inhibits endogenous PGI_2-mediated but not the PGE_2-mediated dilation of coronary arterioles" resulting in "detrimental effects on the bioavailability of two important endothelium-derived factors, NO and PGI_2, for vasodilation" (Hein, Qamirani, Ren, & Kuo, 2009, p. 199).

Vascular Permeability

A number of studies (Mark, Trickler, & Miller, 2001)(Farmer, Girardot, Lepage, Regoli, & Sirois, 2002) (Murohara, et al., 1998) (Farmer, Bernier, Lepage, Guillemette, Regoli, & Sirois, 2001) (Tanita, et al., 1997) investigated how substances such as bradykinin, vascular endothelial growth factor, platelet activating factor, tumor necrosis factor, and mechanically stimulated leukocytes stimulate an increase in vascular permeability. All of these studies were done in vitro and all used a cyclooxygenase inhibitor (indomethacin, ibuprofen, or meclofenamate) to determine effects of a stimulated release of prostaglandins from endothelial cells. The presence of a cyclooxygenase inhibitor was found to decrease the permeability of the endothelial cells in all of these studies.

Inflammation

Reiss & Edelman, (2006) reported that prostaglandins, in part, mediate the inflammatory response in the vascular endothelium. Prostaglandins that are derived from the arachidonic acid, cyclooxygenase pathway are over-expressed in many chronic inflammatory diseases (Maldve, Kim, Muga, & Fischer, 2000). Multiple studies investigated the role of prostaglandins and how they modulate the inflammatory response. Studies also evaluated the role of prostaglandins in rheumatoid arthritis (McCoy, Wicks, & Audoly, 2002), sepsis induced cardiomyopathy (Mebazaa, et al., 2001), chronic pancreatitis (Sun, Reding, Bain, Heikenwalder, Bimmler, & Graf, 2007), chronic rhinosinusitis (Perez-Novo, Claeys, Van Cauwenberge, & Bachert, 2006), and pain from inflammation (Beloeil, Gentili, Benhamou, & Mazoit, 2009) (Bar, et al., 2004). All of these authors reported that the production of PGE_2 is increased during these before mentioned inflammatory states.

In the lungs, inflammatory responses result in an increased production of prostaglandin D_2 (PGD_2), not PGE_2 (Fujitani, Kanaoka, Aritake, Uodome, Okazaki-Hatake, & Urade, 2002). In fact PGE_2 , when given during an asthma related response of inflammation in the airways of mice, was observed to attenuate the allergen-induced inflammation response (Herrerias, et al., 2009).

The inflammatory responses modulated by eicosanoids (prostacyclin, thromboxane, and prostaglandin) have also been implicated in the production of atherosclerotic lesions (Schonbeck, Sukhova, Graber, Coulter, & Libby, 1999). Schonbeck, Sukhova, Graber, Coulter, & Libby, (1999), Belton, Byrne, Kearney, Leahy,

& Fitzgerald, (2000) studied the role of COX-1 and Cox-2 and the production of eicosanoids and atherosclerosis. The authors found that both COX-1 and COX-2 contribute to the increase in PGI_2 production in human atherosclerotic states. Schonbeck, Sukhova, Graber, Coulter, & Libby (1999) reported that COX-1 and COX-2 were expressed in human endothelial cells, smooth muscle cells and macrophages. Kharbanda, et al., (2002) hypothesized that aspirin protected the arterial endothelium from inflammation through inhibition of vascular prostanoid synthesis or from modulation of the inflammatory response. These authors reported that aspirin did not reverse induced endothelial dysfunction. They reported the protective effects of aspirin are from modulation of the cytokine cascade (Kharbanda, et al., 2002).

Sapirstein, Saito, Texel, Samad, O'Leary, & Bonventre, (2005) and Maldve R. E., Kim, Muga, & Fischer, (2000) attempted to understand what substances regulate the COX-2 modulated inflammatory response to $PGE_{2.}$ Maldve, Kim, Muga, & Fischer (2000) reported that the production of COX-1 and COX-2 are up-regulated by their products, prostaglandins. Sapirstein, Saito, Texel, Samad, O'Leary, & Bonventre, (2005) executed a study that examined the role of cytosolic phospholipase as a regulator of COX-2 levels in the brain. These authors reported that cytosolic phospholipase does regulate COX-2 levels, modulating the inflammatory PGE2 levels resulting in a possible pathway that could be inhibited to elicit anti-inflammatory effects.

Indomethacin

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that inhibits prostaglandin synthesis "Prostacyclin (PGI_2) is an unstable prostaglandin which inhibits platelet aggregation and serotonin release and causes vasodilation" (Weksler, Ley, & Jaffe, 1978). Indomethacin has been studied in relation to monolayers, vessels, organs, and whole body effects.

Monolayers

Cultured human endothelial monolayers where stimulated to produce prostacyclin and then exposed to indomethacin resulting in an abolishment of prostacyclin production (Weksler, Ley, & Jaffe, 1978). Corneal endothelial monolayers were also studied because of the significant affects that multiple diseases and trauma can have on the cornea resulting in loss of visual acuity (Joyce & Meklir, 1994). Corneal monolayers repair themselves by migration (Joyce & Meklir, 1994). It was reported that exposing corneal monolayers to indomethacin significantly decreased individual cell migration (Joyce & Meklir, 1994).

Other studies used cultured bovine endothelial cells. Mark, Trickler, & Miller, (2001) exposed bovine brain microvessel endothelial cells to tumor necrosis factor-α (TNF-α) resulting in increased permeability. The cells were then exposed to indomethacin and a significant reduction in permeability was observed (Mark, Trickler, & Miller, 2001). Farmer, Bernier, Lepage, Guillemette, Regoli, & Sirois, (2001) used cultured bovine aortic endothelial cells that were exposed to bradykinin which increased

their permeability. The aortic endothelial cells were then exposed to indomethacin which decreased their permeability by 1.8 fold (Farmer, Bernier, Lepage, Guillemette, Regoli, & Sirois, 2001).

Vessels

A complication of a patent ductus arteriosus may be life threatening for an infant (Jegatheesan & Ianus, 2008). Jegatheesan & Ianus, (2008), Van Overmeire, et al., (1995), Schmidt, Davis, Modderman, Ohlsson, Roberts, & Saigals, (2001) and Van Overmeire, Smets, Lecoutere, Vandebroek, Weyler, & Degroote,(2000) reported that treatment with indomethacin will close the ductus arteriosus, in fact, indomethacin is considered standard therapy. The effects of indomethacin on retinal and choroidal blood flow have also been investigated. Indomethacin was reported to decrease retinal blood flow by 29% and choroidal flow by 17% (Weigert, Berisha, Resch, Karl, Schmetterer, & Garhofer, 2008). Renal (Mizutani, et al., 2009) and uterine (Mints, Luksha, & Kublickiene, 2008) (Milling Smith, Jabbour, & Critchley, 2007) perfusion were also reported to be decreased in the presence of indomethacin. Indomethacin was also observed to decrease the dilation of endothelial cells in the aortas of mice (Viswambharan, et al., 2007).

Organ

The effect of indomethacin on multiple organs of the body has also been well documented. Indomethacin was reported to be ineffective in decreasing the accumulation of fluid in the retina following cataract surgery (Uckerman, et al., 2005). However,

indomethacin was observed to decrease the sequellae of neuronal damage to rabbits after spinal cord trauma (Pantovic, Draganic, Erakovic, Blagovic, Milin, & Simonic, 2005). After an ischemic stroke, indomethacin was observed to enhance the accumulation of newborn cells in rats (Hoehn, Palmer, & Steinberg, 2005). Lozano, Wu, Bassuk, Kurlansky, Lamas, & Adams, (2008) conducted a post cardiopulmonary resuscitation (CPR) study of bovines. The authors reported that indomethacin attenuated the increase in blood flow in the heart and brain, and that prostaglandins have a critical role in the viability of myocardial function during hypotensive states. Indomethacin has also been implicated in the inhibition of cell growth, specifically osteoblasts (Arpornmaeklong, Akarawatcharangura, & Pripatnanont, 2008). In fact, indomethacin is used to prevent heterotopic ossification in acetabular fractures (Blokhuis & Frolke, 2009). Preterm labor is the second leading cause of neonatal death; the release of prostaglandins can stimulate labor (Witcher, 2002). Indomethacin is therefore used as a tocolytic to block the release of prostaglandins. Fortson, et al., (2006) reported that vaginally administered doses of indomethacin were more effective than oral doses. The effect of indomethacin during peritonitis was also investigated to learn if it blocked inflammatory mediators such as vascular endothelial growth factor (VEGF) (Leypoldt, Kamerath, & Gilson, 2007). These authors observed a decrease in the permeability of the peritoneum but not a decrease in the amount of VEGF found (Leypoldt, Kamerath, & Gilson, 2007). Enuresis, or bedwetting, has also been treated with indomethacin. Studies have reported indomethacin to be more effective than a placebo but less effective than the traditional medication, desmopressin (Kamperis, Rittig, Jorgensen, & Djurhuus, 2006).

Whole Body Effects

NSAIDs including indomethacin can be effective in the relief of pain and inflammation (Hung, Chen, Hsu, Chiu, & Chen, 2002). Woo, Man, Lam, & Rainer, (2005) reported indomethacin was safe and effective in treating pain from blunt injury. Sustained release indomethacin was reported to be effective in decreasing post-operative consumption of morphine in patients recovering from a lumbar laminectomy (Rowe, Goodwin, & Miller, 1992). Reimer-Kent, (2003) used indomethacin in a study of post cardiac surgery patients, in conjunction with morphine, and reported decreased length of stay days. However, Hung, Chen, Hsu, Chiu, & Chen, (2002) observed that the reduction in pain sensation may result in increased muscle injury. Indomethacin has also been shown to effectively treat headaches alone (Dodick, Jones, & Capobianco, 2000) or in combination with other medications (Sandrini, et al., 2007). Gout is a painful, acute inflammatory response to deposits of uric acid (Ostrov & Kase, 2004). Indomethacin has been reported to be effective in the treatment of pain from acute gout (Man, Cheung, Cameron, & Rainer, 2007) (Boss, 2007).

It is intersting to note that indomethacin has been studied in relation to other diverse pathologes such as diabetes, alzhiemers disease, and cancer. Avogaro, et al., (1996) studied indomethacin in the presence of disease such as diabetes. These authors reported that indomethacin blocked the production of prostaglandins decreasing the hemodynamic variations found during diabetic ketoacidosis. Sung, et al., (2004) reported there has been some speculation that Alzheimer's disease may be the result of an inflammatory processes from the release of prostaglandins. However, a study using

indomethacin to block the production of prostaglandins reported no significant decrease in the symptoms of Alzheimer's (Sung, et al., 2004).

NSAIDs including indomethacin have also been investigated as a possible treatment for various types of cancer (Loveridge, MacDonald, Thoms, Dunlop, & Stark, 2008). In the presence of colorectal and breast cancer there was an indomethacin-mediated growth inhibition and apoptosis (Loveridge, MacDonald, Thoms, Dunlop, & Stark, 2008) (Markaverich, Crowley, Rodriquez, Shoulars, & Thompson, 2007) (Van Wijngaarden, et al., 2007).

Gaps in Research

Aird, (2009) reported endothelial cells compose the inner lining of all vasculature, including arteries, veins, and capillaries, resulting in a surface area of four thousand to seven thousand square meters. Therefore, the study of endothelial cells includes all organ systems and functions. Endothelial cells have diverse functions and activities regulated differentially by location and time; as a result they have been labeled heterogenetic (Aird, 2009). The heterogeneity of endothelial cells results in a cell that is "highly adaptive and tightly coupled to its own specific microenvironment" (Aird, 2009). These properties are best realized in intact, functioning vessels (Aird, 2009). Frangos, Eskin, McIntire, & Ives, (1985), Waters, (1996), and Tsao, Lewis, Alpert, & Cooke, (1995), investigated endothelial release of prostaglandins as a response to a shear stress stimulus on cultured cells. Aird, (2009, p.2) reported that studies conducted using cultured cells, during in vitro studies, "must be interpreted with caution and ultimately validated in vivo."

The similarities between the mesenteric vessels of frogs and mammals (Bundgaard & Frokjaer-Jensen, 1982) and the development of the Landis technique allows for the study of intact endothelial cells. Williams, (1999), Williams, (2003), and Kim, Harris, & Tarbell, (2005) investigated the effect of shear stress on endothelial cell in relation to hydraulic conductivity and Williams, (2007) studied the effects of pronase on L_p. However, no studies were found that investigated whether indomethacin would decrease L_p of endothelium after a shear stress stimulus.

CHAPTER 3

METHODS

Setting and Sample

The experimental protocols in this study were performed by Donna A. Williams PhD. The methods used were as follows.

Setting

The experimental protocol was completed in the Capillary Physiology and Microcirculation Research Laboratory at the University of Missouri-Columbia, Columbia, MO and at Montana State University, Bozeman, MT.

Sample

All handling procedures and the housing of the animals were approved by the Animal Care and Use Committees at the University of Missouri-Columbia and at Montana State University, Bozeman, MT. The North American leopard frog was used in this experiment. The frogs were caught in the wild and all the frogs used in the experiment were male. Upon arrival to the facility the frogs were bathed in a gentamicin water bath. They were maintained in a temperature controlled environment at 15 degrees C. and were exposed to 12 hours of daylight and 12 hours of darkness. They were kept in an aquarium where they had free access to water and dry areas. The frogs were fed liver baby food every 2 weeks. All frogs had fasted for 4 days prior to testing.

Experimental Methods

A description of the experimental methods follows. The experiment was performed by Donna Williams PhD and published in Microvascular Research January 2007.

Animal Preparation. Each frog was pithed cerebrally and cotton was placed in cranial cavity. A right lateral incision was made through the skin and abdominal wall. Blood samples were collected for measurement of protein concentration (Bio-Rad, Richmond, CA), hematocrit (micro-capillary reader, IEC, Needham Heights, MA), hemoglobin (Drabkins Reagent, Sigma, St. Louis, MO), red blood cell counts, and white blood cell counts (bright line hemacytometer, Hausser Scientific, Horsham, PA). A loop of intestine was exteriorized and coaxed gently around a polished quartz pillar to maintain blood perfusion to the tissue. The mesentery and intestine remained moist and cool (12 to 16˚C) by air-equilibrated frog Ringer's solution flowing continuously across the tissue. Temperature was monitored with a thermocouple wire (type-T, Digi-Sense®, Cole Parmer, Vernon Hills, IL) anchored under the tissue.

Capillary Identification. Transilluminated sections of mesentery were inspected for capillaries using an inverted, compound microscope (UM 10x long working distance objective, 0.22 NA, Diavert, Leitz). Microvascular segments with approximately 500 to 1000um between branch points were defined as individual capillaries. Capillaries were devoid of vascular smooth muscle cells, contained flowing frog red blood cells (fRBC) and had no rolling or sticking WBC. At the branching ends of each capillary the direction

of fRBC flow was noted and each capillary was categorized (Chambers and Zweifach, 1944). TC (true capillaries with divergent flow at one end and convergent flow at the other end) and VC (venular capillaries with convergent flow at both ends) were used in this study.

Video Imaging. The image of each capillary was displayed on a video monitor (PM-125, Ikegami Tsushinki Co., JN) with a miniature high resolution CCD camera (TM-7CN, PULNiX America Inc., Sunnyvale, CA) coupled to the microscope. The camera was equipped with an electronic shutter controller (SC-745) operating at a speed of 1/10,000 s, which allowed either fRBC or human red blood cells (hRBC) to be visualized clearly. Experiments were video recorded in real time (Panasonic AG-6300, Matsushita Electric Industries, JN) with a video timer (0.01 s, VTG-33, For-A, JN) superimposed onto the image of the capillary (final Magnification, 500x). Clips of video recorded data were digitized (media converter DVMC-DA2, Sony) and archived on an external hard drive (LaCie, Ltd.). The scale used for all measurements was calibrated to a stage micrometer (0.01 mm, Meiji Techno, JN).

Glass Microtools. Micropipettes, occluders, and restraining rods were created with a pipette puller (Model PB-7, Narishinge, Tokyo, JN) from acid-rinsed, borosilicate glass (1.5mm, TW150-4, World Precision Instruments, WPI, Sarasota, FL). Each micropipette was ground to a single bevel (12 to 20 um tip OD) using a Narishige grinding wheel (Model EG-4, 0.3 um abrasive film, Thomas Scientific, Swedesboro, NJ). A sealed, acrylic chamber (WPI) held each micropipette in position and a water

manometer was attached to the chamber to maintain pressure and flow. Three micromanipulators (Prior, Stoelting Co., Wood Dale, IL) were used to adjust the glass microtools in the X-Y-Z planes.

Solutions. Frog Ringer's was prepared fresh daily from 5x concentrated stock solution (pH 7.4 and 15 degrees C) and contained (in mM) NaCl (111.0), KCl (2.4), MgSO4 (1.0), CaCl2 (1.1), glucose (5.0), NaHCO3 (2.0), and N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES)/Na-HEPES (5.0). Dialyzed (6000 to 8000 MWCO, Spectra/Por membrane, Spectrum, Houston, TX) bovine serum albumin (BSA, 10mg ml-1; A-4378, Sigma Chemical co., St. Louis, MO) dissolved in frog Ringer's was used as the control solution. hRBC were obtained from a finger prick, washed 3 times in Ringer's, and added to each pipette solution (2 to 3% vol/vol hematocrit). All solutions were maintained on ice until use and visible bubbles were removed when each micropipette was back-filled with solutions.

Mechanical Stimulus. Abrupt change in shear stress and shear stress time curves- Each capillary was cannulated at 8cm H_20 (5.9 mm Hg, *in situ* TC pressure) and low flow was established for 2 min (Steady State 1). Next, manometer pressure was changed abruptly to 30 cm H20 (22.1 mm Hg) to produce the mechanical stimulus, change shear stress. The higher flow was maintained for 2 min (Steady State 2) at which point the capillary was occluded to measure volume flux/surface area (J_v/S) also at 30 cm H2O. It was assumed that filtration during occlusion reflected filtration at Steady State 2. A time curve for shear stress was generated for each capillary from measures of the free-flowing

hRBC. Plateaus for Steady State 1 and Steady State 2 were identified by visual inspection from these curves. The magnitude of the mechanical stimulus (change shear stress) was calculated from the plateau values as: change in shear stress=shear stress (Steady state 2)- shear stress (Steady State 1) (1).

The intent of each experiment always was to induce a square wave stimulus; however, deviations from intent do occur with work *in situ*. As such, the actual stimuli were categorized into square wave, overshoot, or undershoot patterns. As described previously (Williams, 2003), a square wave designation was assigned when *t* at 15 s was within 2.0 dynes cm $^{-2}$ of the mean value for Steady State 2. Capillaries were excluded from the change t/L_p analysis due to technical problems, which included overshoot or undershoot stimulus patterns and/or interruption of Steady State 2 by a clogged pipette.

Estimates of Capillary Fluid τ From Measures of RBC
Velocity (v), Capillary Diameter, and Apparent Viscosity.

The method used to measure v of RBC has been described in detail (Williams, 1999, 2003). Briefly, instantaneous RBC velocity (v^i) was measured directly on the video monitor in the frame-by-frame mode as the distance traveled by a single hRBC in time (d*x*/d*t*, *u*m s-1). $v^i = dx/dt$ (2)

A correction factor (CF; Michel et al., 1974) was calculated from hRBC radius (R, *u*m) and each capillary radius (r, µm, measured simultaneously with v^i) as: $F=2(1-[(R)^2/2(r)^2])$ (3)

v was then calculated from v^i and CF assuming a centered RBC within the vessel. v(µm s-1)=vi/CF (4)

Capillary diameter- Diameter was measured from video recording at 3 sites spaced about 100 μm apart along the capillary and averaged.

Apparent viscosity- $n^{apparent}$ was estimated from protein concentration (frog plasma, before, and BSA/Ringer's, after cannulation; Chick and Lubrzynska, 1914) as, $n^{apparent}$(poise)=(5.7x 10-5[protein, mg ml^{-1}]^{37degreesC}) + 0.0089), (5) and corrected to 15degrees C using the ratio between viscosity of water at 37 and 15 degrees C (West, 1970). Use of protein concentration provided an estimate for the lower limit of capillary fluid viscosity.

t- Shear rate was calculated from v and r as: Shear Rate(s^{-1}) =4v/r. (6) t was then calculated as: t(dynes cm^{-2}) = (Shear Rate)($n^{apparent}$). (7)

Calculations of volume flux (Jv) per surface area (S) and hydraulic conductivity (Lp) from measures of capillary diameter and Jv.

Jv- The modified Landis technique (Landis, 1927; Michel et al., 1974) was selected to measure Jv to ensure that the downstream end of each capillary remained intact prior to and during the mechanical change in shear stress stimulus. After the change in shear stress protocol a glass occluder, which had been positioned over the downstream end of the capillary perpendicular to the longitudinal axis of the microvessel, was lowered gently onto the capillary to occlude it and trap the hRBC. Movement of the trapped hRBC acted as an index of fluid flux across the capillary wall. Visual inspection verified successful occlusion. Time course data were obtained from serial occlusions, all at 30cm H_2O, moving the occluder progressively upstream along the capillary towards

the micropipette, with one J_v measurement per occlusion and minimum of 12 μm between each occlusion site (Williams, 2007. p 49-50).

Experimental Protocols.

Two vessels from each frog were cannulated; one was used as a control and the other had indomethacin (fresh stock solution of indomethacin 10-5M**)** superfused over the tissue for 5 to 7 minutes prior to assessment of Lp. Once the vessel was cannulated, low flow was maintained for two minutes and then the abrupt $\Delta\tau$ protocol was performed. Human RBC's were then infused through the pipette and measurements were obtained. All experiments were performed by the same individual to minimize variance in mechanical manipulation.

Data Collection for Thesis.

All measurements of RBC velocity, capillary diameter, and J_v were performed by the thesis investigator with the assistance of Mary H. Flood, lab assistant to Dr. Williams, who is responsible for quality control. Ms. Flood taught the thesis investigator the protocols as follows: 1) I developed a measuring scale that was calibrated to the stage micrometer (0.01mm, Meiji Techno, JN); 2) Distortion was eliminated from the monitor screen; 3) a tape was marked 0-60 microns, with large marks at increments of 5 and small marks at increments of 1; 4) The 0 on the tape measure, was placed at the junction of the end of the screen and the end of the vessel being measured. Ms. Flood observed this investigator performing the measurements and compared this thesis investigators findings with the quality control findings.

This investigator then used the video images to perform the required measurements so that Lp could be calculated. Figure 3 illustrates a frame of the video used for performing the measurements. This investigator did have the opportunity to perform cannulation of a capillary following one of Dr. Williams' experiments.



Figure 3. Video Frame of Frog Capillary that is Cannulated and Human Red Blood Cells are Infused. Courtesy Dr. Donna A. Williams.

At the time the original data were collected, each video of two experimental protocols performed by Dr. Williams were labeled. The video tapes used by this investigator were randomly selected from the two protocols and another masters thesis

investigator randomly selected another set of videos. As such, each student investigator performed measurements on 15 individual experiments and each was blind to which protocol was used. After the measurements were performed Dr. Williams sorted the data by experiment, one data set was from frogs who demonstrated signs of inflammation the other data set was from frogs who did not have an indication of inflammation. This investigator received the data set obtained from frogs that did not show signs of infection.

The measurements and calculations performed on each vessel were as follows. The first was capillary diameter. Diameter was measured 3 times at 3 time points during occlusion of the capillary to verify that, within the limits of detection and resolution of the video system (2μm), *S* remained constant. Capillary volume and *S* were calculated from capillary radius (*r*, cm). A cylindrical capillary geometry was assumed (Williams, 2007).

J_v/S- J_v/S was calculated from hRBC velocity measured during the occlusion ($dx/dt^{occlusion}$, cm s^{-1}), capillary length measured between the occluder and each marker cell at the onset of the dx/dt measurement (x_o, cm), and the volume to surface area ratio ($r/2$, cm) (Williams, 2007).

$J_v/S(\text{cm s}^{-1})=(dx/dt^{occlusion})(1/x_o)(r/2) - dx/dt^{occulsion}$ and x_o were measured on 3 hRBC (spaced ≈50 μm apart) at 3 time points (2.0,2.3, and 2.6s). Thus, 3 individual values of J_v/S were obtained from the first occlusion to decrease the standard deviation of the mean and increase precision (Williams, 2007).

L_p- Assuming a linear relationship between J_v/S and capillary pressure (P_c) as per the Starling equation, the slope obtained from regression analysis of J_v/S and P_c was used to estimate capillary filtration coefficient, L_p (cm s^{-1} cm H_20^{-1}) (Williams, 2007).

$J_v/S=L_p[(P_c-P_i) – \sigma(\pi c – \pi i)]$ where (Pc-Pi) and ($\pi c – \pi i$) are the differences in hydrostatic (P) and oncotic (π) pressures between the capillary lumen (c) and interstitium (i) of the mesentery and sigma (σ) is the reflection coefficient of the capillary barrier to protein. With an exteriorized mesentery, interstitial pressure is negligible and with fresh, protein-free frog Ringer's solution superfusing the tissue continuously, oncotic pressure differences are difficult to estimate. Calculating interstitial pressure from mesenteric protein concentration altered values for L_p by 0.1 x 10^{-7} cm s^{-1} cm H_2O^{-1}, a difference that was within the error of the method (Williams, 2007).

Statistical Analyses

Data reported in tables and texts are means±SE unless otherwise indicated. Data were analyzed by using a box plot to assess for normal distribution and outliers, four outliers were removed from the treatment group. All P-values were calculated using a paired, 2 tailed students t-test, used to evaluate differences in means. Median Lp P-values was calculated using the Wilcoxon signed rank test. A P-value of 0.05 was considered significant and was set prior to commencing experimental protocols.

CHAPTER 4

RESULTS

Descriptive measurements of the frogs (n = 16) are listed in Table 1. These measurements were taken prior to experiments. A control capillary and an experimental capillary were cannulated in each frog. Table 2 contains means of measurements performed prior to cannulation of both the control and experimental capillaries. Measurements of diameter and velocity were needed to calculate Lp. Shear rate and shear stress were measured to assure the same amount of stimulus was used to evoke responses from the capillary. Tube hematocrit (hct) was measured to evaluate the amount of chronic shear stress in the vessel. There was not a statistically significant difference between the pre-cannulation control and experimental capillaries for diameter, mean velocity, shear rate, shear stress, or tube hematocrit (hct). Appendix A contains tables of the actual measurements from all of the frogs and vessels.

Table 1. Descriptive Measurements

	Mean (SD)
Frog Wt. (g)	29.1 ±5.6
Length (cm)	18.5±1.1
Heart Rate (bpm)	68.1±6.6
Hemoglobin (g/dl)	7.1±1.8

Table 1. - Continued. Descriptive Measurements

Hematocrit	25.8±7.8
Total WBC's cells/mm³	4776.5±2242

Table 2. Pre-cannulation Measures Performed on Two Capillaries Per Frog

	Control Capillary	Treatment Capillary	P-value
Diameter (µm)	12.9±2.9	12.0±1.1	0.27
Mean Velocity (µm/s)	672.2±295	584.5±264.5	0.39
Shear Rate (s-1)	425.5±166.4	404±190.8	0.74
Shear Stress (dynes·cm^{-2})	7.1±2.8	6.7±3.4	0.73
Tube hct (%)	10.2±5.0	13.3±7.7	0.18

After cannulation of the vessels the measurements of diameter, mean velocity, shear rate, and shear stress were taken again. The mean diameter did not vary greatly between pre-cannulation control vessel (12.9±2.9) and post-cannulation control vessel (13.2±2.1). However, there was a statistically significant post-cannulation increase in diameter mean when comparing control and treatment capillaries. Balance pressure is the pressure at which a steady state is established between the capillary flow and the flow of the micropipette. There was a significant difference in balance pressure between control and treatment vessels (see Table 3).

Shear stress is used as a stimulus to provoke a response of prostaglandin release from the endothelium. As a result of the shear stress stimulus experimental protocol, marked changes in the measured means of velocity, shear rate, and shear stress are noted when comparing pre- and post-cannulation of the capillaries. The increase in means occurred both in control and experimental vessels which resulted in a non-statistically significant difference between control vessels and experimental vessels (see table 3). The change in shear stress was measured to ensure that the same amount of stimulation was used in each vessel. The change in shear stress was not statistically significant (P=0.66) between control and treatment groups, see table 4.

Table 3. Post-cannulation Measures Performed on Two Capillaries Per Frog

	Control Capillary	Treatment Capillary	P-value
Diameter (μm)	13.2±2.1	14.7±2.2	0.04
Mean Velocity (μm/s)	4447.3±1231	4908.5±1025	0.26
Shear Rate (s-1)	2753.5±884	2543.2±410	0.42
Shear Stress (dynes·cm^{-2})	42.9±13.8	39.7±6.4	0.42
Balance pressure (cmH20)	10.4±1.8	8.3±1.7	0.002

Table 4. Capillary

	Control	Treatment	P-value
Δ in shear stress	37.2±13.4	42.1±16.3	0.66
Lp (mean)	3.5 ±1.7	2.5 ±1.7	0.13
Lp (median)	3.4	1.8	0.08

Following the stimulation of the capillary, using shear stress, and superfusion of indomethacin, Lp was measured. In comparing the control and treatment capillaries, there was not a statistically significant difference in Lp (P=0.13), see Table 4. A scatter plot showing Lp as a function of change in shear stress (Δτ) is shown in figure 4. The scatter plot illustrates considerable overlap between the control and treatment data sets consistent with no difference between average and median values for Lp.

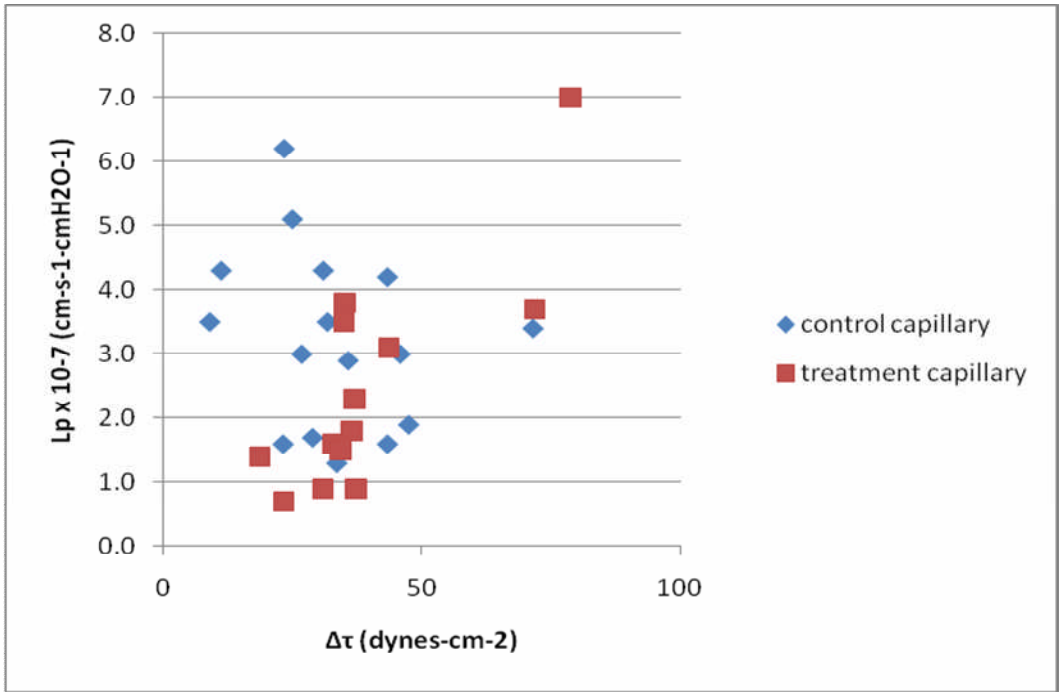


Figure 4. Scatter Plot of Lp Graphed as a Function of the Magnitude of a Change in Shear Stress for Control and Indomethacin Treated Capillaries.

CHAPTER 5

DISCUSSION

Cultured human endothelial cells release prostaglandins after a shear stress stimulus, prostaglandins increase $L_{p,}$ and indomethacin is known to mediate the effects of prostaglandins (Cotran, Kumar, & Collins, 1999) (Frangos, Eskin, McIntire, & Ives, 1985). Therefore, we hypothesized that indomethacin should decrease capillary L_p following a shear stress challenge. Data from this study was contrary to the hypothesis that indomethacin decreases Lp of capillaries located in healthy frogs.

Given that L_p did not decrease in the presence of indomethacin, it is probable that other processes are effecting the response of the capillary endothelium to a shear stress stimulus. For example the endothelium could be releasing NO simultaneously resulting in a buffered response to the shear stress stimulus and the infusion of indomethacin. An additional possibility is that another component of a chemical mediator may need to be present for indomethacin to affect Lp. A further possibility is that the endothelium responds differently in vitro when it is cultured in a petri dish versus in vivo endothelium in an intact capillary. The study published by Frangos, Eskin, McIntire, & Ives (1985) used cultured human endothelial cells and evaluated the rate at which the endothelium released PGI_2 in response to a shear stress stimulus. They indicated that the rate of PGI_2 production from the endothelium varied depending on the type of endothelial cells (arterial vs. venous) and if the cells were cultured under physiologic flow conditions.

Perhaps, there is a constituent of the intact capillary that exerts some control over the release of PGI_2 that would not be seen when using cultured endothelial cells.

Priebe (2010) conducted a similar study where mesentery capillaries from the North American leopard frog were used to evaluate the effects of indomethacin on Lp. The capillary bed was superfused with indomethacin and then individual capillaries were cannulated by Dr. Williams. A shear stress experimental protocol identical to the one used in the present study was followed to stimulate the endothelial lining of the capillary. The capillary was occluded at a point distal to cannulation. The rate of the hRBC from the cannulation pipette and through the capillary was measured and Lp was calculated. The only difference between the present study and that of the Priebe study was the frogs showed signs of inflammation in the Priebe study. Priebe reported a statistically significant decrease in Lp when the frogs showed systemic signs of inflammation and the capillaries were superfused with indomethacin (Priebe, 2010). This would suggest that inflammation must be present for indomethacin to have a significant effect on the release of prostaglandins from the capillary endothelium. When comparing the Priebe study with this study there is a possibility that the frogs that showed signs of inflammation already had capillaries with a higher Lp than the frog without signs of inflammation. The control capillaries in the Priebe study had a mean Lp of 10.9, in this study the mean Lp was 3.5. Evoking a response from the endothelium of frogs with signs of inflammation by a shear stress stimulus decreased the Lp when indomethacin was introduced. The capillaries in this study were not inflamed and, as such, did not have the rate of capillary leak that the inflamed capillaries displayed.

The goal of Dr. Williams' research has always been to advance the science of capillary physiology. Knowledge of endothelial function has progressed from being recognized as a mere barrier, to understanding that endothelium functions as a regulator of physiologic responses to varied stimuli. Increasing the understanding of capillary and endothelial cell responses and functions has tremendous potential to increase the understanding of multiple disease processes and potentially open new avenues of treatment for many diseases. The data from this study demonstrated that indomethacin did not alter capillary function following a shear stress challenge. However, the data, especially the scatter plot appears to show a tighter grouping of the treatment Lp when compared to the control Lp.

Clinical Significance

In the clinical setting NSAIDs are used as antipyretics, analgesics, and anti-inflammatories for the management of pain from multiple chronic and acute diseases including: rheumatoid and osteoarthritis, gout, bursitis, and headaches. They are also prescribed for post-operative pain and certain ophthalmic inflammatory processes (Setter, Corbett, Sclar, Gates, & Johnson, 2001). It is estimated that 60 million prescriptions are written for NSAIDs yearly and approximately 3.6 times more for elderly patients than for the young (Solomon D. H., 2009a). More than 17 million Americans use at least one of the twenty different forms of NSAIDs available in the United States resulting in millions of dollars spent annually (Solomon D. H., 2009a).

The majority of patients and many health care providers believe that NSAIDs are 'safe,' however; five to seven percent of hospital admissions are a result of adverse drug effects and thirty percent of those are related to NSAID use (Solomon D. H., 2009a). NSAIDs can be toxic to the gastrointestinal (GI), renal, cardiovascular, and hepatic systems, often from the inhibition of prostaglandin production (Solomon D. H., 2009a). Gastrointestinal effects include dyspepsia, peptic ulcer disease, and bleeding (Solomon D. H., 2009b). Individuals using NSAIDs chronically and the elderly are at the highest risk for developing GI toxicity (Song & Marcon, 2009). Song and Marcon, (2009) also report that two-thirds of NSAID users showed signs of intestinal inflammation; a second study indicated that patients who developed intestinal perforation were twice as likely to have consumed NSAIDs (Song & Marcon, 2009). The documented and reported increasing evidence of frequent adverse GI effects of NSAIDs led to the development of COX-2 inhibitors. They do not inhibit the production of prostaglandins to the extent of combined COX-1and COX-2 inhibitors in the GI system (Moore, Derry, Phillips, & McQuay, 2006).

Acute renal failure from either renal ischemia or interstitial nephritis often with nephrotic syndrome can also be a result of NSAID toxicity (Rose & Post, 2007). In healthy individuals prostaglandins do not effect renal perfusion; however, in the presence of glomerular disease prostaglandin production is increased. Inhibiting the production of prostaglandin can result in renal ischemia (Rose & Post, 2007). The mechanisms through which NSAIDs induce acute interstitial nephritis have not been fully defined, but these

patients often present with hematuria, pyuria, white cell casts, protienuria, and elevated creatinine (Rose & Post, 2007).

NSAIDs affect the cardiovascular system in multiple ways including interfering with the ability of aspirin to bind to protein, decreasing the cardio-protective effects of aspirin (Solomon D. H., 2009a). Cardiovascular risks for myocardial infarction and stroke increases with the use of some nonselective and most selective NSAIDs (Solomon D. H., 2009a). In fact an advisory was published by the American Heart Association expressing the need to carefully weigh the pros and cons of prescribing selective NSAIDs, including the patient's cardiovascular risks (Bennett, Daugherty, Herrington, Greenland, Roberts, & Taubert, 2005). The American Heart Association also advised consumers of over-the-counter NSAIDs to follow the product labels closely and if NSAIDs were used longer than ten days consumers should consult their health care provider (Bennett, Daugherty, Herrington, Greenland, Roberts, & Taubert, 2005).

In 2004 rofecoxib (Vioxx), a selective NSAID was removed from the market. The removal of this drug from the market stimulated continued scrutiny of all NSAIDs (O'Malley, 2006). Recent research revealed a higher incidence of MI, sudden cardiac death, ischemic stroke, and unstable angina in patients taking COX-2 inhibitors (O'Malley, 2006). Researchers have also found that patients taking nonselective NSAIDs have an increased risk of having a cardiovascular event and the Federal Drug Association has requested a "black label warning" be placed on all of these products (O'Malley, 2006).

The prevalence of heart failure is estimated from three-tenths of a percent to twenty percent of the general public and is steadily increasing due to the increased MI survival rate (Bleumink, Feenstra, Sterkenboom, & Stricker, 2003). Patients with heart failure have a five year mortality rate of forty percent creating a need to identify risks for exacerbation (Bleumink, Feenstra, Sterkenboom, & Stricker, 2003). In patients with heart failure, an exacerbation of this condition may be induced with the use of NSAIDs because of the vasoconstricting effects that can result in an increased afterload (Solomon D. H., 2009a). With the development of COX-2 inhibitors it was hoped, that besides decreasing the GI effects of NSAIDs, renal sparing properties would also be noted (Bleumink, Feenstra, Sterkenboom, & Stricker, 2003). However, there seems to be production of both COX-1 and COX-2 enzymes in the kidneys resulting in no change in the incidence of nephrotoxicity (Bleumink, Feenstra, Sterkenboom, & Stricker, 2003). Consequently it has been suggested that NSAIDs should be prescribed to patients with heart failure sparingly (Bleumink, Feenstra, Sterkenboom, & Stricker, 2003). If the benefits of treatment with NSAIDs are found to outweigh the risks, then renal function should be monitored closely and the patient should be closely monitored for signs of heart failure. Patients should also be thoroughly educated about symptoms of adverse effects (Bleumink, Feenstra, Sterkenboom, & Stricker, 2003).

The vast use and accessibility of NSAIDs, the perception that they are ‘safe’ medications and the potentially devastating toxic effects of NSAIDs should compel providers to prescribe them cautiously and responsibly. Having a solid understanding of NSAIDs physiologic effects is one of the best ways of accomplishing responsible

prescribing. This study investigated indomethacin, which is in the class of NSAIDs. Findings in this study are specific to indomethacin and should not be generalized to all NSAIDs. However, when comparing data from this study with findings from the study by Priebe (2010), new physiologic evidence revealed that an element of inflammation must be present in order for NSAIDs to have an effect on prostaglandin release.

NSAIDs are often used to treat the pain symptoms of osteoarthritis when acetaminophen has failed (Kalunian, 2008). However, osteoarthritis has two distinct categories, inflammatory and noninflammatory (Kalunian, 2008). This study suggests that NSAIDs may not be effective for the noninflammatory types of osteoarthritis. The results of this study cannot be generalized to anything other than the North American leopard Frog. However, when you take into account this physiologic evidence and the potential toxic effects of NSAIDs, further investigation appears to be necessary.

Many forms of NSAIDs are sold over-the-counter (OTC). The public often views medications that are accessible without a prescription as having few risks. Health care providers, drug companies, and government agencies must develop a plan to educate the public of the potentially toxic effects NSAIDs. Health care providers also must be aware of patients who are taking OTCs such as NSAIDs or acetaminophen when prescribing narcotics. Many prescription narcotics have an NSAID or acetaminophen in combination with the narcotic. Patient education should also address the concern that taking other OTC medications may contain the same drugs as the prescribed combination pain medication, and could consequently result in toxicity, overdose, or even death. Patient education must be thorough because a large number of consumers are unaware of the

relationship and differences between generic and brand name medications. Patient education should also address the issues associated with self-diagnosis and self-medication with OTC medications. It is understandable that patients do not want to return to their provider for every eruption of gout or other reoccurring ailments. However, patients must understand the inherent risks of self-diagnosing and self-medicating. Patient education must include information that just because medications are available OTC does not mean they are without hazards.

This research revealed a glimpse of the importance of capillary endothelial cells, how they function as a separate entity, controlling multiple physiologic responses within the body. Endothelial dysfunction is the basis for multiple disease processes such as diabetes, atherosclerosis, heart failure, and hypertension. Paramount to understanding disease processes is a detailed understanding of how endothelial cells function, what triggers their responses, and how they communicate with other cells. Certainly, an improved understanding of the disease process will result in improved management and decreased morbidity and mortality from these diseases.

Understanding that NSAIDs are effective in decreasing Lp in the presence of inflammation, but are not effective in the absence of inflammation, has clinical implications in many medical conditions that result in capillary leakage. The loss of fluid from the capillary, into the tissue, causing edema can be the result of many different pathological conditions including heart failure, renal failure, liver disease, sepsis, pancreatitis, and other infectious syndromes (Aneja & Carcillo, 2009). Often many of these conditions can result in depleted intravascular fluid or hypovolemia, causing

hypotension and decreased oxygenation to tissue. Patients diagnosed with sepsis, such as the one described in the introduction are treated with large amounts of fluid and vasopressors if fluid volume is not adequate (Schmidt & Mandel, 2009). These patients may benefit from the use of an NSAID to decrease the loss of fluid from the capillaries (Priebe, 2010). However, this study would indicate that the use of NSAIDs in conditions when inflammation is not present such as heart failure, renal failure, or liver disease would not decrease capillary leak or edema.

Indications for the use of NSAIDs are inflammation and pain (Solomon, D. H., 2009b). Pain is often the result of inflammation. NSAIDs decrease inflammation and pain by blocking the production of cyclooxygenase which inhibits the production of prostaglandin (Solomon, D. H., 2009b). The release of prostaglandins results in increased capillary leak or increased Lp. The frog capillaries used in this study showed no indication of inflammation and Lp was not decreased after the superfusion of indomethacin. Therefore, this study suggests there is need for further study of the indications for use of NSAIDs.

Science, such as physiology, and the scientific method are not owned by any one discipline. Individuals in the nurse practitioner role are well suited to take on the challenge of integrating bench science with disease processes seen in their practices. The profession of nurse practitioners is encouraged to embrace bench science as an integral component of its discipline. This will not only provide nurse practitioners with an understanding of the rigor of scientific method and the evidence that supports interventions, treatments and pharmacotherapeutic management of disease but it will also

validate the credibility of nurse practitioners as scholars, scientists, and health care providers. Today, healthcare is dictated by guidelines and recommendations. Individualized practitioner interventions, based on an integration of practice guidelines and a formidable understanding of disease physiology, will result in improved outcomes and increased quality of life.

Recommendations for Further Research

When assessing the data of this study and the study of Priebe (2010) it was noted that signs of inflammation is the only differing variable. A study by Priebe (2010) reported a decrease in Lp, when indomethacin was superfused and signs of inflammation were present. However, this study did not find a decrease in Lp in the treatment vessels which suggests further research into indications for use of NSAIDs. Further investigations into the release of prostaglandins from intact healthy capillaries after a shear stress stimulus are needed, as well as investigations into inflammation and how inflammation affects the release of prostaglandins after a shear stress stimulus in intact capillaries. The effect of a shear stress stimulus on the endothelial cells of capillaries has implications for many disease processes including; diabetes and diabetic neuropathy, cardiovascular disease, hypertension, renal disease and wound healing.

Non-steroidal anti-inflammatory drugs are often used to treat pain that results from a variety of disease processes. The public often believes that most NSAIDs are safe and have minimal side effects. However, the toxic effects of NSAIDs have high morbidity and mortality rates especially in the elderly (Solomon, D.H., 2009a). The easy

availability of NSAIDs may lead individuals to self-medicate and to take unnecessary and potentially harmful medication. It is extremely important that NSAIDs are used appropriately to treat specific pathologies. This study, using indomethacin, suggests there may be evidence at the cellular level that NSAIDs do not alter capillary function following a fluid shear stress stimulus. It is unknown if NSAIDs other than indomethacin would produce the same results. Further investigation into the correlation of the effectiveness of NSAIDs and the presence of inflammation is needed. The study should be repeated with other NSAIDs that are commonly used for acute pain, muscle strain and headache such as ibuprofen, naproxen, and salicytic acid.

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APPENDIX A:

RAW DATA

Table 5. Description of Frogs

Control	Experiment Date	Time (hr:min)	Frog Wt. (g)	Length (cm)	HR (bpm)	hb (g/dl)	Total WBC's cells/mm^3	Vender
IndoTC08	2/14/06	14:07	31.2	18.4	64	8.1	4000	Tennessee
IndoTC12	2/21/06	10:25	27.9	17.8	72	7.0	4800	Tennessee
IndoTC17	3/21/06	14:31	25.5	17.4	54	6.1	6800	Wisconsin
IndoTC20	3/24/06	10:34	18.7	15.9	66	5.2	2400	Wisconsin
IndoTC22	4/14/06	10:34	23.8	18.1	74	6.6	3200	Wisconsin
IndoTC45	5/15/07	10:22	38.2	20.9	82	11.7	2800	Tennessee
63Indo/PG12	7/25/07	10:28	32.4	19.3	73	7.1	4400	Tennessee
IndoTC02	1/27/06	10:59	34.6	19	70	9.8	4000	Tennessee
IndoTC05	2/7/06	13:41	27.7	18.5	68	7.4	6000	Tennessee
IndoTC07	2/10/06	13:50	25.8	17.8	65	8.4	2800	North Dakota
IndoTC09	2/16/06	10:28	35.6	18.8	59	5.4	3200	Tennessee
IndoTC27	5/10/06	13:43	19.1	17.5	72	5.6	3200	Wisconsin
IndoTC46	5/15/07	13:50	29.4	19.7	69	9.0	4400	Tennessee
IndoTC41	8/22/06	13:34	29.3	18.8	66	5.0	11200	Tennessee
IndoTC31	6/14/06	11:18	27.5	19.1	75	7.1	8000	Tennessee
IndoTC03	1/27/06	14:03	36.2	19.2	67	6.5	5600	Vermont
IndoTC04	2/7/06	10:41	31.6	18.3	62	5.3	4400	Tennessee

Table 6. Pre-cannulation Measurements for Control Vessels

Control	Diameter (μm)	Mean Velocity (μm/s)	Shear Rate (s-1)	Shear Stress (dynes·cm^{-2})
IndoTC08	17.0	657	309.2	5.3
IndoTC12	12.3	483.0	313.3	5.1
IndoTC17	13.0	1139	700.9	11.3
IndoTC20	10.7	331.2	248.4	3.8
IndoTC22	15.0	1165.2	621.4	10.9
IndoTC45	10.0	761	608.8	10.2
63Indo/PG12	12.5	4102.8	2602.7	44
IndoTC02	15.0	420.4	224.2	3.9
IndoTC05	13.6	683.0	411.7	6.5
IndoTC07	12.0	775.5	517.0	8.6
IndoTC09	12.5	930.1	620.1	10
IndoTC27	16.8	3019.3	1472.3	24
IndoTC46	14.0	840.8	480.4	7.0
IndoTC41	7.0	427.9	489	8.1
IndoTC31	18.0	911.2	405	7.0
IndoTC03	9.6	254.7	208.8	3.7
IndoTC04	10.8	302.5	223.6	3.6

Table 7. Pre-cannulation Measurements for Experimental Vessels

Indomethacin Treated	Diameter (μm)	Mean Velocity (μm/s)	Shear Rate (s-1)	Shear Stress (dynes·cm^{-2})
IndoTC08	12.7	506.7	319.9	5.5
IndoTC12	11.3	543.0	383.3	6.3
IndoTC17	13.0	200.4	123.3	2.0
IndoTC20	11.7	421.7	289.2	4.4
IndoTC22	12.0	591.7	394.5	6.9
IndoTC45	12.0	293	195.3	3.3
63Indo/PG12	19.5	1894.8	780.1	13.2
IndoTC02	13.0	1004.7	618.2	10.9
IndoTC05	10.7	282.9	212.2	3.3
IndoTC07	16.0	820.3	410.1	5.3
IndoTC09	13.0	794.4	488.8	7.9
IndoTC27	14.5	753.6	420.5	6.9
IndoTC46	10.7	577.5	433.1	7.3
IndoTC41	11.5	1035.9	727.1	12.3
IndoTC31	11.7	259.9	180.3	3.1
IndoTC03	10.8	824.5	605.9	10.7
IndoTC04	12.0	442.3	294.9	4.8

Table 8. Post-cannulation Measurements for Control Vessels

Control	Diameter (μm)	Mean Velocity (μm/s)	Shear Rate (s-1)	Shear Stress (dynes·cm^{-2})
IndoTC08	17.0	4354.3	2049.1	32.0
IndoTC12	14.0	5706.7	3261.0	50.9
IndoTC17	15.0	5166.2	2755.3	43
IndoTC20	10.7	6436.8	4827.6	75.3
IndoTC22	13.0	3543.4	2180.6	34.0
IndoTC45	12.0	4413.7	2942.4	45.9
63Indo/PG12	12.0	6589.3	4392.9	68.5
IndoTC02	14.0	5299.6	3028.4	47.2
IndoTC05	12.0	5180.1	3453.4	53.9
IndoTC07	13.0	3897.3	2398.4	37.4
IndoTC09	12.0	5122.3	3414.9	53.3
IndoTC27	12.0	4802.1	3201.4	49.9
IndoTC46	15.0	3972.1	2118.4	33
IndoTC41	10.5	3510.9	2678.8	41.8
IndoTC31	18.0	4377.3	1945.5	30.3
IndoTC03	11.1	3086.3	2216.8	34.6
IndoTC04	11.3	1138.7	803.8	12.5

Table 9. Post-cannulation Measurements for Experimental Vessels

Indomethacin Treatment	Diameter (μm)	Mean Velocity (μm/s)	Shear Rate (s-1)	Shear Stress (dynes·cm^{-2})
IndoTC08	17.0	5787.7	2723.6	42.5
IndoTC12	15.0	5146.2	2744.6	42.8
IndoTC17	14.0	9065.9	5180.5	80.8
IndoTC20	12.3	3680.5	2387.4	37.2
IndoTC22	13.0	3936.7	2422.6	37.8
IndoTC45	16.7	5016.0	2407.7	37.6
63Indo/PG12	20.7	5290.1	2047.8	31.9
IndoTC02	13.0	4787.8	2946.4	46.0
IndoTC05	12.0	7232.1	4821.4	75.2
IndoTC07	17.0	3892.2	1831.6	28.6
IndoTC09	16.0	5794.3	2897.1	45.2
IndoTC27	13.0	4364.5	2685.9	41.9
IndoTC46	15.0	6133.6	3271.2	51.0
IndoTC41	14.0	4654.3	2659.6	41.5
IndoTC31	15.0	3529.9	1882.6	29.4
IndoTC03	14.0	13310.7	7606.1	118.7
IndoTC04	13.0	4382.3	2696.8	42.1

Table 10. Change in Shear Stress After Stimulus (dynes·cm^{-2})

	Control	Indomethacin Treatment
IndoTC08	28.5	39.6
IndoTC12	43.2	39.9
IndoTC17	37.3	70.7
IndoTC20	68.7	33.4
IndoTC22	28.1	35.5
IndoTC45	43.9	33.7
63Indo/PG12	62.7	29.9
IndoTC02	29.5	44.2
IndoTC05	46.9	67.2
IndoTC07	30.6	23.3
IndoTC09	44.7	36.0
IndoTC27	48.4	34.8
IndoTC46	30.9	49.0
IndoTC41	39.8	33.3
IndoTC31	24.5	25.4
IndoTC03	29.7	81.6
IndoTC04	7.8	37.8

Table 11. Balance Pressure (cm H20) (%)

	Control	Indomethacin Treatment
IndoTC08	9.6	
IndoTC12	8.6	7.1
IndoTC17	8.0	7.2
IndoTC20	7.4	7.4
IndoTC22	12.6	9.2
IndoTC45	11.2	8.5
63Indo/PG12	11.7	6.4
IndoTC02	13.5	10.3
IndoTC05	9.2	7.5
IndoTC07	9.8	10.9
IndoTC09	8.9	6.1
IndoTC27	13.5	10.4
IndoTC46	10.4	8.0
IndoTC41	9.8	11.2
IndoTC31	12.2	7.4
IndoTC03	11.5	6.1
IndoTC04	8.6	8.6

Table 12. Tube hct (using ellipse)

	Control	Indomethacin Treatment
IndoTC08	9.1	19.8
IndoTC12	19.0	23.1
IndoTC17	12.0	14.2
IndoTC20	13.4	5.0
IndoTC22	9.0	19.8
IndoTC45	25.5	18.8
63Indo/PG12	4.3	7.2
IndoTC02	8.7	7.1
IndoTC05	8.9	26.0
IndoTC07	14.6	6.4
IndoTC09	12.8	11.5
IndoTC27	4.0	6.3
IndoTC46	9.9	14.0
IndoTC41	10.7	12.8
IndoTC31	4.2	1.7
IndoTC03	19.5	25.7
IndoTC04	2.6	6.8

(# of cell's in 500micron length in the measured capillary- T-tests between capillaries-same)

Table 13. Lp (cm/s/cmH_20)

	Control	Indomethacin Treatment
IndoTC08	3.0	2.3
IndoTC12	3.0	1.8
IndoTC17	3.5	7.0
IndoTC20	3.4	1.6
IndoTC22	1.6	0.9
IndoTC45	2.9	1.5
63Indo/PG12	4.3	1.4
IndoTC02	1.6	3.8
IndoTC05	1.9	3.7
IndoTC07	1.7	0.7
IndoTC09	4.2	11.4
IndoTC27	7.4	3.5
IndoTC46	5.1	3.1
IndoTC41	1.3	7.8
IndoTC31	6.2	23.0
IndoTC03	4.3	14.0
IndoTC04	3.5	0.9