The development of a translational model for the analysis of microvascular failure in sepsis

Medical Biophysics 3970Z

Rachel McInnis

Instructor - Ian MacDonald

TA - Nate Hayward

April 10, 2013
The development of a translational model for the analysis of microvascular failure in sepsis

RACHEL L. MCINNIS
Department of Medical Biophysics, Western University, London, Ontario, Canada

INTRODUCTION

Sepsis is the leading cause of death in North American intensive care units [6]. In Canada (outside Quebec), there was a 14% increase in the number of cases of sepsis hospitalization from 2004-2005 to 2008-2009 [7]. Of these cases the majority of patients were older adults and young children with a median patient age of 66 [7]. Pre-existing co-morbidities such as cancer and diabetes were present in a higher number of septic patients [7]. The mortality rate for all sepsis patients was 30.5% in Canada (excluding Quebec) which makes up approximately one-tenth of all hospital deaths [7]. Examination of figures in the United States has revealed that patient prospects are highly similar to those in Canada [1]. The severity of sepsis coupled with the fact that anyone may develop sepsis has motivated research efforts to gain basic knowledge of its impact on the microcirculation.

The cardiovascular system is essential for the transportation of nutrients and waste throughout the body. One element of this system, the microcirculation controls the delivery of red blood cells (RBCs) and plasma within the organs [2]. In sepsis, however, this function is disrupted resulting in irregularities in the microvasculature perfusion in vivo [3]. Sepsis is the systematic inflammatory response resulting from bacterial infection which is characterized by the failure of one or more organ systems [6]. It is particularly difficult to diagnose because of variability in symptoms [7,9].
The diversity in pathophysiological pathways adds an element of complexity in developing treatments [9]. Further complications arise due to the lack of specificity in the diagnostic criteria of sepsis [7, 9]. It is necessary to develop effective treatments to improve patient outcome prospects and to minimize the costs on the healthcare system. It is especially important to find treatments given the aging population because of the prevalence of sepsis in the elderly. This experiment aims to improve upon past studies to develop a translational animal model of sepsis for analysis of a capillary network. Previous studies have been unsuccessful because they have inaccurately modelled the clinical circumstances. A peritonitis model of sepsis in rats will simulate an intensive care patient through monitoring physiological parameters. Supportive therapies will also be provided sepsis through employing mechanical ventilation and fluid resuscitation to better mimic the clinical situation. Based on the knowledge of microvasculature in sepsis [3], a difference in perfusion of the capillary bed at a site remote to injury should be observed after the induction of sepsis over time.

**THEORY**

Krogh [10] first proposed a model to describe oxygen transport in the capillaries in striated muscle. The model idealized the microcirculation of muscle tissue as one capillary cross-section providing oxygen to a surrounding cylindrical portion of tissue. This is an important model to consider when analysing the microvasculature irregularities of sepsis because a change in the microvasculature will alter oxygen supply to the tissue. The geometry and the distribution of RBC flow in a vascular network are integral to its function which is known to be altered in sepsis [3]. The Krogh model [10] describes that a certain oxygen pressure is required to adequately supply oxygen to the tissue cylinder.
RBCs facilitate the transportation of oxygen in the circulatory system which aims to distribute it to tissue. There are two different forms of hemoglobin: oxyhemoglobin (HbO$_2$) and deoxyhemoglobin (Hb) which enable this function. The binding of oxygen to Hb to form HbO$_2$ results in a change in the molecular extinction coefficient. At the 442 nm wavelength HbO$_2$ and Hb exhibit different extinction coefficients; therefore, it is considered an oxygen sensitive wavelength. The 454 nm wavelength is an isobestic point which is a wavelength where the absorption coefficients are equal [4]. Additional isobestic points are shown for Hb and HbO$_2$ at locations on the graph presented in Figure 1 where the curve of Hb and HbO$_2$ intersect with one another. To calculate oxygen saturation video sequences must be recorded at an isobestic wavelength and an oxygen sensitive wavelength. RBC oxygen saturation is calculated by software which involves a spectrophotometric technique that uses RBC optical density as described by Fraser et al. [5].

Fig 1 Molar extinction coefficient of Hb and HbO$_2$ as measured by Prahl (11).
In addition to oxygen saturation, the video sequences may be used to collect information pertaining to the hemodynamics of the capillaries. From the analysis of a single capture from the video recording RBC flow cannot be discerned; therefore, it is necessary to process the videos to gain insight on the distribution of RBC flow in the microvasculature. A pixel in any given video frame has an intensity value \( I(x,y) \) which varies from 0-255 for 8-bit gray scale [8]. \((x,y)\) indicates the position of a pixel within a frame. The variance of intensity value is

\[
V(x,y) = \frac{1}{N-1} \left[ \sum_{i=1}^{N} I_i(x,y)^2 - \frac{1}{N} \left( \sum_{i=1}^{N} I_i(x,y) \right)^2 \right]
\]

(1)

where N denotes the number of frames. Variance images are sensitive to differences in lineal density; however, they cannot detect changes in velocity of the RBCs [8]. This means that capillaries in focus with RBC flow will exhibit a higher variance and thus appear brighter in the functional image. On the other hand capillaries which are not within the plane of focus or have limited blood flow will have lower variance and appear darker. The variance images are a visualization tool for a comparative analysis of the RBC flow in the microvasculature [8]. Sum in the absolute difference (SAD) images are a similar type of image which will be used in this study to analyse the perfusion of a capillary network after the induction of sepsis across time. These images typically display a greater quantity of vessels than variance images.

**METHODS**

**Animal Preparation**

Male Spargue-Dawley rats (100 g) were kept in an animal storage facility until they had grown to a suitable mass between 180 and 210 g. The animals were randomized to three experimental groups: sham laparotomy, (sham; n=1), fecal injection into the peritoneum (FIP;
n=1), and FIP treated with fluid resuscitation (FIP+fluid; n=1). The experimental protocol was approved by the University of Western Ontario’s Animal Care and Use Committee.

The animals were anesthetized before performing the surgical procedure with pentobarbital (65 mg/kg body weight). The right carotid artery was cannulated to measure mean arterial pressure and heart rate. The left jugular vein was cannulated to administer supplementary anesthetic and saline through the course of the experiment. Fluids were given to all three experimental groups; however, the rate that the fluids were administered was varied between groups. Animals were mechanically ventilated through insertion of a tracheal tube (33% oxygen). Heat lamps were employed to maintain the animal’s core temperature at 37.0°C. The temperature was monitored using a rectal temperature probe.

The FIP procedure involved preparing a fecal solution comprised of the animal’s feces and saline. The fecal solution (1 mL/kg body weight) was subsequently injected into the animal’s peritoneal cavity. In both the sham and FIP group 0.9% saline was administered at a rate of 0.5 ml/hr. In the FIP treated with fluid resuscitation this rate was increased to 0.25 ml/hr.

**Extensor digitorum longus muscle (EDL)**

The *extensor digitorum longus* (EDL) muscle of the hind limb was exposed and the tendon was separated from the bone. The animal was transferred to the stage of the microscope to allow for transillumination of the muscle. The exposed muscle was held at the *in situ* length using a ligature with the lateral side facing the objective. The muscle was covered with a glass slip and saran wrap to prevent oxygen diffusion with the environment. Warmed saline was regularly transferred onto the muscle and surrounding tissue to prevent desiccation.
**Functional Microvascular Imaging**

The microvasculature of the rat EDL muscle was observed using intravital video microscopy (IVVM) at 10 X magnification. After inducing sepsis, 5 minute videos were taken hourly at a rate of 21 frames/sec at one field of view. The videos were captured with a QImaging Rolera XR (QImaging, Surry, BC, Canada) fitted with a 100W Xenon lamp. A beam splitter contained interference filters at 442 nm and 454 nm. A monitor was used to display the live video sequences at both wavelengths using two identical computer systems. Before each recording the microscope was refocused and repositioned to ensure capture of the same field of view. A process similar to creating the functional image is described by Japee et. al. (8).

**RESULTS**

![A. Image capture from a video taken at 10X magnification of the microvasculature in the rat EDL muscle. B. Processed image from a 5 minute video sequence of the same capillary bed shown in (A). In the SAD image shown the capillaries with RBC perfusion appear as bright in the image while the vessels with less perfusion appear darker.](image-url)
Fig. 3 Functional images of a capillary network in rat skeletal muscle in the sham animal model taken through the duration of the experiment. The level of perfusion of the vessels in the field of view remained relatively constant from 1 hour to 5 hours after the completion of the surgical procedure.

Fig. 4 Captures taken at 1 hour, 3 hours, and 4 hours after the induction of sepsis using the FIP procedure. The captures are functional images of the same capillary bed in the EDL muscle in the septic animal model. A decrease in capillary perfusion from 1 hour to 3 hours and 3 hours to 4 hours is visualized in the SAD images by the increase in the dark regions within the field of view. This is indicative of stop-flow capillaries remote to the initial site of injury.
Fig. 5 SAD images of the rat EDL muscle in the FIP treated with fluid resuscitation model at 1 hour, 2 hours, 3 hours, 5 hours and 10 hours. The functional images visualize a decrease in capillary perfusion up to 3 hours after the induction of sepsis. After fluid resuscitation at 3 hours through a five-fold increase in the fluid administration to the animal an increase in RBC flow through the capillary network is shown. Regions of the vasculature that were initially perfused and then subsequently dysfunctional at 3 hours were again showing RBC flow after 10 hours. Flow also improved from intermittent to steady after fluid resuscitation.

**DISCUSSION**

The processing of the video sequences taken in the rat EDL muscle allow for flow visualization of the capillary network. The sham group did not exhibit the microvascular failure seen in the FIP model of sepsis. The functional images of the FIP model showed that vessels that were initially perfused later became stopped flow vessels. This increase in stopped flow capillaries is consistent with the findings of previous studies [2, 3]. The capillary blockages seen in the FIP sepsis model are likely the result of stiffening of the leukocytes and RBCs as well as
fibrin/platelet clots [2]. If the quantity of perfused capillaries is decreased then each of the capillaries with flow remaining would be responsible for providing oxygen to a greater volume of tissue than they had prior to the induction of sepsis. The shift towards a greater prevalence of stopped flow capillaries observed in sepsis has been shown to decrease the minimum tissue oxygen tension decreases in mathematical models [6].

The FIP treated with fluid resuscitation group showed steady flow of RBCs through the capillary bed in the EDL muscle of the rat. This restoration in capillary perfusion may be explained by increasing plasma volume [2]. Further work to determine the best time for fluid resuscitation and the optimal quantity of fluid to be administered is an important step in establishing which treatment provides the greatest outcome. Supplying fluids to septic patients is a part of early goal-directed therapy which has been shown to be beneficial [12].

The images provide a window into the microvasculature in the skeletal muscle after an injection into the peritoneal cavity which triggered sepsis. This implies that other remote sites would also exhibit dysfunction in the microvasculature. The exact characteristics of this dysfunction are unknown due to the unique geometries in the microcirculation of different organs. Other studies have suggested that the change in the characteristics of blood flow as seen in sepsis results in a disparity in oxygen supply to oxygen demand in the tissue [6].

The analysis of functional images involved a qualitative comparison; however, it would be necessary to complete quantitative analysis to draw further conclusions. This process would involve calculating functional capillary density as well as calculating RBC oxygen saturation. The videos collected through this experiment already contain the necessary information to determine these parameters. This model also opens the possibility for creating a clinically relevant model of sepsis which would require including the expertise of physicians to better
simulate the conditions of an intensive care unit in a hospital. Successfully modelling sepsis is an essential first step in the investigation of possible treatments.

CONCLUSION

The functional images in the rat EDL muscle for the sham, FIP, and FIP treated with fluid resuscitation models allowed for analysis of changes in blood flow in the capillary network. The sham animal exhibited consistent perfusion of the microvasculature over a 5 hour period after the completion of the surgical procedure. The FIP animal showed a decrease in perfusion of the capillary bed within 5 hours. The FIP treated with fluid resuscitation animal displayed similar blood flow in the microvasculature as the FIP model until fluid resuscitation given after 3 hours. Treatment with fluid resuscitation restored capillary perfusion and resulted in steady flow of RBCs through the capillaries. Overall, this study has built upon existing knowledge of the pathophysiology of sepsis through utilizing SAD images to provide a window into the microvasculature.

ACKNOWLEDGEMENTS
I would like to thank Dr. Chris Ellis for his guidance and Stephanie Milkovich for her assistance in the animal preparation. I would also like to thank Nathaniel Hayward for performing the experiment and capturing a portion of the video sequences. This research was supported by Canadian Institutes of Health Research Grant MOP-102504 (to C. G. Ellis).

REFERENCES


