Exploring the hepatic microcirculation in critical illness: a challenge

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Abstract. A substantial number of patients admitted to the ICU with a major infection develop sepsis or septic shock. The role of the liver and hepatic microcirculation in subsequent events, including organ failure, has been established but continues to be a topic of intensive research. Combined effects on contractile sinusoidal cells i.e. endothelial cells and stellate or Ito cells, regulate the hepatic arterial and venous portal parts of the perfusion in the liver sinusoids with consecutive effects on hepatocyte function and metabolism. However, improving inadequate liver function, based on deficient tissue perfusion and oxygenation, and assessing therapeutic interventions by monitoring the liver microcirculation is difficult. Current monitoring techniques include liver-specific clearance of substances and intravital microscopic visualization of the microcirculation combined with computer-aided video analysis. Simple and minimally invasive bedside monitoring of the microcirculation would be a great advantage in the future.

Introduction

One of the most important goals of therapy in critically ill patients is to restore and maintain adequate perfusion and oxygenation of vital organs during recovery from circulatory failure, such as in sepsis and trauma.

Microvascular dysfunction early in sepsis represents the first critical stage in tissue hypoxia and organ failure [1]. The gastrointestinal tract plays a key role in the development of shock and multiple organ failure. The impaired integrity of the mucosal layer when rendered hypoxic disrupts the barrier function against luminal bacteria and bacterial products like endotoxins [2, 3]. Translocation of bacteria and endotoxins to the lymphatic and portal systems occurs initially and leads ultimately to distant organ damage. The gut and liver macrophages (Kupfer cells) are important as a first line of defence against further spread to the general circulation. Mesenteric perfusion and autoregulation of the microcirculatory blood flow distribution in the splanchnic area is impaired during systemic bacteraemia and in endotoxaemia. Regional blood flow distribution is flawed and changes under normal circumstances are not reflected in conditions of sepsis and septic shock. The flow distribution to the mucosa is decreased in sepsis while the general splanchnic flow remains largely intact [2, 4].

Blood flow distribution and regional perfusion have to adapt to local metabolic demand. Because the diffusion of oxygen in tissues is limited, the nutritional delivery has to depend on a dense, finely distributed network of capillaries [1, 5].

The microcirculatory blood flow in the splanchnic organs is heterogeneous both in early hypodynamic shock as well as later in hyperdynamic shock, and can therefore not be predicted by general changes in systemic or even regional blood flow [6]. Consequently, monitoring the microcirculation seems to be important in our aim to identify patients at risk of developing organ system failure, and to evaluate our therapeutic interventions [1, 7-9]. Although hepatic dys-

function is known to independently influence outcome and recovery of critically ill patients and seems especially warranted in daily practice, due to the anatomical particulars, measuring the hepatic circulation poses a unique challenge [10, 11]. Unlike any other organ, the liver receives blood from two types of afferent vessel i.e. the portal system and the hepatic artery, and monitoring of this system at bedside is difficult at present, except perhaps at laparotomy.

This article presents an overview of some physiological aspects of the hepatic microcirculation and oxygenation, the different methods used to monitor hepatic perfusion and oxygenation, and their relevance in the research and the clinical setting.

Specifics of hepatic anatomy

Together with the small bile duct culminating in the common bile duct, the terminal portal venules and the terminal hepatic arterioles form a triad which forms the basis of the microscopic structure of the liver. The hepatocytes are organized in the Kiernan’s or classic lobules around central hepatic venules, which form the hepatic vein (in a hexagonal outline), the boundaries of which are defined in humans by imaginary interlobular septal lines. The portal tracts (composed of terminal portal venules, terminal hepatic arterioles and the smallest branches of the bile duct) are situated in the ‘corners’ of the hepatic lobules [12, 13]. Functionally, a further refinement is the ‘simple liver acinus’, consisting of the smallest portal tract at the centre and the terminal hepatic venule in the periphery. The zone closest to the afferent vessel is referred to as ‘zone 1’ and the area surrounding the peripheral efferent hepatic venule as ‘zone 3’. ‘Zone 2’ is located between these two areas (Fig. 1).

This morphological and functional structure provides a rational approach to study the hepatic metabolism in each zone because, for instance, an oxygen gradient exists from zones 1 to 3, making zone 3 hepatocytes vulnerable to oxygen deprivation as in sepsis [14]. The cytochrome P-450 3A4 system (e.g. essential for drug metabolism) is located predominantly in zone 3 hepatocytes and is oxygen dependent or even hypoxic, as was shown in mice [15]. Compromised flow will reduce its function and lead to accumulation of the primary drug.
The portal venous system (terminating in the portal venules) and the hepatic arterial system derived from the celiac trunk (forming terminal hepatic arterioles) come together in the liver capillary bed, called the sinusoids [12, 16]. The hepatic arterial blood flows indirectly into the sinusoids via an anastomosis between the terminal hepatic arteriole and the portal venule, but also directly into the sinusoids [16, 17]. Regulation of the blood flow into the sinusoids is maintained by the relaxation and contraction of a precapillary sphincter at the end of the terminal hepatic arteriole, and also by the coordinated contraction and dilatation of the sinusoidal endothelial fenestrae around the portal tract zone and by regulators in the portal venous system [17]. Furthermore, the stellate or Ito cells have an important regulatory role.

**Physiological aspects of hepatic microcirculation**

The terminal hepatic arterioles supply oxygen-enriched blood to the bile ducts, the walls of the portal venules as vasa vasorum, and to the nerves and connective tissues, and in addition to the portal system play a significant role as nutrient vessels [12, 17]. Blood flow in the sinusoids examined by intravital video-enhanced contrast microscopy was found to be significantly slower (400-450 µm/sec) than in normal or ‘true’ capillaries (500-1000 µm/sec), thus facilitating the metabolic exchange of sinusoidal blood with the hepatocytes. The diameter of the sinusoids was smaller and the blood flow velocity was slower in zone 1 than in zone 3; this may indicate a difference in metabolism or structure [17]. Endothelins as vasoconstrictors and nitric oxide (NO) as a vasodilator are derived from endothelial cells and are considered to be mandatory for the local regulation of the microcirculation in the liver [12, 13, 18]. Endothelin administration results in a constriction of the portal venules and terminal portal venules, comparable to the effects of norepinephrine. The sieving plates or fenestrae of the sinusoids contract, as do the terminal hepatic arterioles directly connected to the sinusoids, both contributing to an overall elevation of portal pressure [17].

The role of the vegetative nervous system has not been clarified entirely and is the subject of intensive research. Parasympathetic branches from the vagus nerve and sympathetic branches derived from the celiac ganglion innervate the liver. Although sympathetic and parasympathetic influence on the liver blood flow has not been established, excitation of sympathetic nerves leads to a general reduction in flow [13]. Complete denervation in liver transplants results in an almost complete loss of epinephrine and norepinephrine concentrations in the liver, but the effects on the hepatic microcirculation in the liver [12, 16]. The hepatic arterial blood flows indirectly into the sinusoids [12, 16]. Flow and oxygen gradient are indicated by arrows.

**Cellular function in hepatic microcirculation**

Disse’s space is situated between the sinusoidal endothelial cells and the hepatocyte surface. Fenestrated cell processes and the absence of a basement membrane characterize the endothelial cells. The stellate or Ito cells are located in Disse’s space, their long cytoplasmic processes surrounding the sinusoids. Hepatic stellate cells represent 5-8% of all liver cells in humans. On the luminal side of the endothelium lie the Kupfer mononuclear-phagocytic cells and liver-associated lymphatic cells [16]. As mentioned above, smooth muscle cells in portal venules and hepatic venules regulate the pre- and post-sinusoidal vascular resistance. In the sinusoids, the endothelial and stellate cells are regarded as the resistance regulators. Several substances in vitro influence the stellate cell tonus e.g. endothelins, angiotensin II, prostaglandin F₂α and vasopressin are constrictive whereas NO, carbon monoxide and prostaglandin E₂ are dilatory [12, 13]. When stimulated, as occurs in sepsis, stellate cells undergo myofibroblastic transformation with loss of vitamin A and release of pro-inflammatory and pro-fibrinogenetic mediators [23].

The arginine-NO pathway plays an important role in infection, inflammation and organ failure [24]. NO is derived from arginine by normally active endogenous and inducible NO synthases, located in different cell types, but mainly in endothelium and hepatocytes from which the inducible form was first found in humans [25]. NO acts as a vasodilator; it inhibits leukocyte adhesion to endothelium and thrombocyte aggregation. It also regulates immunological cytotoxicity and lymphocyte function.

All liver cells have been reported to express inducible NO synthase (iNOS) [25, 26]. Once expressed, iNOS continually produces NO, in contrast to endogenous NO synthase (eNOS) through which NO is produced ‘on demand’. eNOS in the liver is expressed in the liver by sinusoidal endothelial cells only and exerts a beneficial and cell-protective effect [25, 27]. The role of iNOS is more complex. In cold ischaemia/reperfusion injury iNOS had a cytoprotective, anti-apoptotic effect, but in haemorrhagic shock experiments, selective NO blocking showed a general deleterious effect of iNOS [25].

**Hepatic ischaemia/reperfusion injury**

Ischaemia-reperfusion (IR) injury of the liver occurs in several clinically relevant conditions, such as haemorrhagic shock, liver transplantation, liver resection and sepsis. Interruption of blood flow...
followed by reperfusion leads to significant cellular damage in liver transplants. Experimental evidence indicates that activation of Kupffer cells and T cells after reperfusion mediates the activation of polymorphonuclear cells (PMN), sinusoidal endothelial cells, and the production of reactive oxygen species [25, 28]. In response to I/R, sinusoidal endothelial cells become activated, and on reperfusion express an array of adhesion molecules and MHC antigens, resulting in further PMN interactions and disruption of sinusoidal blood flow. PMN-induced hepatocyte injury results from adhesion of the two cell types, release by the PMNs of toxic enzymes like elastases and metalloproteinases, and induction of oxygen free radical species [28, 29]. The role of NOS in I/R remains controversial: experiments in eNOS knockout mice demonstrated an increase in hepatotoxicity in I/R liver damage, while iNOS was shown to contribute to warm I/R injury, but beneficially influence apoptosis in cold I/R injury as seen in organ transplantation [25]. Thus, the mechanisms by which NO exerts an effect depends on the type of I/R and the source of the NO produced.

Methods of monitoring the hepatic microcirculation

Assessment of the oxygenation and organ perfusion has been studied in numerous experimental and clinical studies. Adequate monitoring tools have been developed and new techniques allow for a better understanding of the changes in the circulation in humans, including the hepatic microcirculation in sepsis and other important clinical settings. In pathological conditions, a general picture of decreased capillary density, increased perfusion heterogeneity and clinical settings. In pathological conditions, a general picture of decreased capillary density, increased perfusion heterogeneity and increased percentages of poorly- or non-perfused capillaries were found [8, 30, 34, 38].

Measuring the hepatic microcirculation is difficult, partly because of the specific anatomy of the hepatic macrocirculation. We will discuss several techniques used in the research and clinical setting below.

Indicator dilution and clearance techniques

One of the main problems with measurements in humans is the inaccessibility of the portal system for haemodynamic studies, and almost all knowledge about the pathophysiological picture e.g. in sepsis, is derived from animal data. Indicator dilution techniques (mainly using 131I-labelled albumin or 51Cr-labelled erythrocytes) require catheterization of the hepatic artery, the portal vein, the superior mesenteric artery or the splenic artery, as well as catheterization of the hepatic vein and are, therefore, very invasive; in addition, due to inaccuracy in portal-systemic shunting, nowadays this technique is almost redundant.

In clearance techniques, several substances have been used to measure hepatic blood flow indirectly. All are based on the principle of a fast and mainly hepatic extraction; in a flow-dependent way the liver removes most of the measured substance, and ideally no other organ system is involved in the plasma clearance. Typically, the intracellular metabolism of the measured substance is not rate limiting in removing the substance from the circulation. The indicator material should ideally be easy to obtain and to administer, be non-toxic, and should not influence metabolism. Older substances included bromosulphthalein, 32P-colloidal chromic phosphate and 14C-taurine. Due to their toxicity, the radioisotope labelling and to the use of more modern techniques, these older methods have been largely abandoned.

Much of the data on hepatic blood flow in humans are based on measurements with indocyanine green clearance (ICG), but others have also been discussed [31-36]. Characteristics for ICG are the negligible extra-hepatic elimination, minimal correction for non-steady-state if the infusion dose is kept low, and a good correlation with direct flow measurements by e.g., Doppler flow. Based on the Fick principle, the plasma level measured in arterial and venous blood with a known constant infusion rate will give the clearance rate of the liver and is representative of liver blood flow. However, the technique has been criticized for its technical and methodological flaws e.g. the fact that the hepatic extraction ratio differs under different clinical circumstances, necessitating invasive sampling from the hepatic vein in the human situation [31, 34, 36].

The metabolism of lidocaine with formation of monoocthyglycine xylidide (MEGX), was used extensively in liver transplant research, but proved to be unsuccessful in septic patients [35, 36]. Our group could not establish a reliable correlation of MEGX formation and flow-dependent lidocaine or midazolam clearance by cytochrome P-450 3A4 in healthy volunteers [37].

D-sorbitol, a natural polyol, is another substance with a potentially ideal profile (non-toxic, high hepatic extraction ratio, easy to measure) for this type of measurement and has been used extensively; however, also here different results have been reported [33, 34, 38].

Physical perfusion measurement techniques

Organ blood flow has been extensively researched with microspheres, direct Doppler and electromagnetic flow probes, and other physical techniques. Isotope labelled microsphere distribution can be used only in terminal experiments, because the liver (or other) tissue has to be sampled and homogenized to measure the radioactivity for flow distribution. Labelling with fluorescents coupled to intravital microscopy improved this technique dramatically, but occlusion of part of the microcirculation increases vascular resistance and tissue hypoxia and has an acute effect on the results [39, 40]. For this reason it can be used to measure the influence of hepatic artery or portal occlusion on the liver microcirculation [36].

Doppler flow probes can only be used to measure hepatic blood flow if the abdomen is opened and besides being very accurate they have been used extensively in the research setting only [8, 41, 42].
Reflection spectrophotometry, based on absorption and scattering of reflected visible light, measures the microvascular haemoglobin oxygenation ($\mu$HbO$_2$), based on the different absorption spectra of oxygenated and deoxygenated haemoglobin. Combined with laser Doppler flowmetry this enables tissue perfusion measurements [43]. It has the advantage of ease of use in different settings, but only an average of $1 \text{ mm}^3$ of superficial organ tissue can be measured, without regard to its morphology, the microcirculatory components and the flow direction. However, it has been extensively used since its introduction in 1979 and, although validation with existing techniques of microcirculation research proved to be difficult, it correlated well with outcome in several settings [44-46].

Orthogonal polarization spectral (OPS) imaging, which can be used for near-surface imaging of the microvascular anatomy, is a further refinement of intravital microscopy. OPS uses polarized green light to illuminate the surface; and by filtering of the reflection allows the flow of blood cells in the microcirculation to be observed, without the need to apply indicator dyes or other image-enhancing substances, as used to be necessary in microvascular research by intravital microscopy [47, 48]. A good correlation between sublingual OPS measurements and development of organ failure and outcome in septic and critically ill patients, has been reported [30, 49]. Spronk et al. showed a therapy-guiding role for OPS in a clinical setting [50]. Intra-operative measurements taken on the liver surface showed microcirculatory alterations predictive for outcome in liver transplant settings [49, 51]. By combining OPS images with semi-quantitative analysis, an assessment of the microcirculation is now possible [52-54].

A significant recent improvement of OPS is known as ‘sidestream dark field’ (SDF) imaging. Less surface reflection interference allows for computer-aided automatic quantification of microcirculatory flow patterns and our group has conducted preliminary studies with OPS on the liver surface [9, 53, 55] (Fig. 2).

Conclusion
Impairment of the microcirculation through endothelial dysfunction, resulting in decreased tissue oxygenation and disturbed cellular and organ function, is generally accepted as pivotal in critical illness. Success in achieving the therapeutic goals of correcting and improving the circulation and meeting tissue oxygen demands correlates with patient outcome. Knowledge of the physiology of the microcirculation in sepsis and other clinically relevant situations is expanding and has proven to be most important in the development and assessment of new treatment strategies. Evaluation of therapeutic interventions on liver function through monitoring of the hepatosplanchnic microcirculation seems feasible, but unfortunately is not feasible to carry out routinely in the setting of everyday ICU practice. Therapy, guided and monitored by the microcirculation emphasizing improved hepatosplanchnic perfusion and oxygenation, is currently only possible in a research setting. Development of a new, simple and minimally invasive technique for bedside monitoring of the microcirculation is necessary and should be encouraged.

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References