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Imaging of the intestinal microcirculation

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Abstract

Microcirculatory dysfunction is important in different intestinal pathologies. Therefore, it is essential for adequate therapeutic strategies to be based on reliable microcirculatory diagnostics. Intestinal microvascular perfusion is regulated by an intricate interplay of neuroendocrine, paracrine and mechano-sensory pathways. While rectal microvascular bed can be readily examined at the patient's bedside, microcirculation of other parts of the gut can only be assessed intra-operatively or by means of enterostomies. Changes in intestinal microcirculation in various diseases, as observed in animal experiments, further contribute to our understanding of intestinal microcirculation in humans. If microcirculatory changes are not adequately taken care of, perfusion will be reduced and tissue oxygenation may be endangered. Relevant clinical studies are presented in this article. Future developments, e. g. miniaturization of optical probes or swallow-able cameras, will facilitate sophisticated diagnostics and thus improve treatment results.

Key words: microcirculation, intestinal, imaging, microcirculatory perfusion

Introduction

All organs are dependent on microvascular perfusion to function and survive, because individual cells need supply of oxygen and nutrients and elimination of waste products via the microcirculation (1). In clinical practice, blood pressure and cardiac output measurements are used for circulatory assessments, but central hemodynamic parameters can be satisfactory in spite of critical insufficiency at the microcirculatory level (2). Sophisticated techniques are required for assessment of microcirculation. With regard to the intestinal microcirculation (IMIC), only the rectal microvascular bed is readily available for human examination at the bedside. The microcirculation of other parts of the gut can only be assessed intra-operatively or via examination of preexisting enterostomies, respectively.

In the present review we summarize intestinal microcirculatory changes in various diseases that can be observed in animal experiments, and provide an overview on the current status of intestinal microcirculation diagnostics in humans. Furthermore, we screened the available literature regarding clinical imaging studies of the IMIC. Finally, we discuss requirements for future developments in intestinal microcirculation research.

Intestinal microcirculatory changes in various diseases

Intestinal microvascular perfusion is regulated by an intricate interplay of neuroendocrine, paracrine, and mechano-sensory pathways. These mechanisms adapt to the balance between locoregional tissue oxygen

transport and metabolic needs to ensure that supply matches demand (3).

Animal experiments have revealed intestinal microcirculatory dysfunction in various diseases. Most drastical changes are observed in sepsis. The gastrointestinal tract plays an important role in sepsis, since disturbances of the intestinal microcirculation can lead to disruption of the mucosa barrier and result in bacterial and toxin translocation into the systemic circulation (gut motor hypothesis, (4)). Several changes have been described within the microcirculation, such as decreased deformability of red blood cells due to an increase in membrane lipid viscosity (5, 6), an increased percentage of activated neutrophils with decreased deformability and increased aggregability due to upregulation of adhesion molecules (6, 7), activation of the clotting cascade with fibrin deposition and the formation of microthrombi (8, 9), and secondary enhanced perfusion of large arteriovenous shunts (10). A characteristic phenomenon of intestinal microcirculatory disturbances in sepsis is the heterogeneity of the changes with some capillaries being normally or even hyper-perfused while others are hypo- or non-perfused with the risk of gut hypoxia (11).

Microcirculation plays an important role in the pathogenesis of inflammatory bowel diseases (IBD). The chronically inflamed endothelium contributes to enhanced leukocyte adhesion (12). In IBD, a hypercoagulable state and a prothrombotic condition is described in the microvasculature (13). Adherence of platelets to the endothelium is a typical event in IBD (14). The enhancement of angiogenesis in IBD highlights neovascularization as a major contributor to the initiation and perpetuation of chronic intestinal inflammation (15).

Bowel ischemia and reperfusion (I/R) and transplantation represent further examples where function of intestinal microcirculation is of high clinical interest. Changes within the microcirculation observed under these conditions are comparable to the above-described changes in acute and chronic inflammation, e.g. leukocyte and platelet adhesion (16).

In summary, all these changes within the intestinal microcirculation can result in reduced microvascular perfusion and may endanger tissue oxygenation.

Clinical imaging of the intestinal microcirculation

The ideal imaging tool to study the intestinal microcirculation in human should be non-invasive, safe, with high sensitivity, specificity and reproducibility, and low costs. However, at present no technology is capable to fulfill all criteria. Imaging modalities like thermography, angiography, computerized tomography or ultrasound have only a limited resolution with regard to the microvasculature. Recently, significant advances were achieved by contrast-enhanced ultrasound (CEUS, (17)). Miniaturized ultrasound probes are available and capable to measure blood flow in small vessels but need invasive insertion (18, 19).

For clinical studies laser Doppler flowmetry (LDF) is frequently used (20) but single-fibre, laser-scanning and laser speckle devices are also available. LDF analyzes superficial tissue blood perfusion without physical contact, dyes, or tracer elements, thus minimizing the influence on perfusion and the risk of contamination, infection, or discomfort to the patient. The more advanced laser-scanning and Laser Speckle Contrast Analysis (LASCA) methods generate a perfusion image of a larger tissue area (21). Microcirculatory perfusion is calculated from the speed and concentration of red blood cells moving through the microvessels.

More recently, mobile microscopical imaging devices were introduced for applied studies of the microcirculation. Miniaturization of the optical and electronical parts made it possible to reduce the device size significantly. The orthogonal polarization spectral (OPS) imaging device was the first handheld device to study the human micro-

circulation in tissues with superficial capillaries (e.g. sublingual, (22)). A second generation handheld device is available now using sidestream dark-field (SDF) imaging technology (www.microvisionmedical.com). The third generation is announced (Braedius Scientific; www.braedius.com) and will provide increased spatial and temporal resolution. The images that can be obtained by these methods are very close to intravital microscopy in experimental animals (Figure 1).

In SDF imaging, illumination is provided by concentrically placed light emitting diodes (LEDs) surrounding a central light guide. The lens system in the core of the light guide is optically isolated from the illuminating outer ring thus preventing the microcirculatory image from contamination by tissue surface reflections. Light from the illuminating outer core of the SDF probe, which penetrates the tissue illuminates the tissue-embedded microcirculation by scattering. The LEDs emit at a central wavelength of 530 nm, chosen to correspond to an isosbestic point in the absorption spectra of deoxy- and oxyhemoglobin (i.e., 530 nm) to ensure optimal optical absorption by hemoglobin, independent of its oxygenation state. This leads to an image where red blood cells (RBCs) are visualized as dark moving globules against a bright background. To improve the imaging of moving structures such as flowing RBCs, the LEDs provide pulsed illumination in synchrony with the charged coupled devices frame rate to perform intravital stroboscopy. This stroboscopic imaging, prevents smearing of moving features, such as flowing RBCs, and motion-induced blurring of capillaries due to the short illumination intervals (23).

With the arrival of modern imaging devices the number of publications regarding human clinical trials with microcirculatory endpoints is just starting to grow (see Table 1). In part excellent studies are already available but several scoring systems have been used so it is sometimes difficult to compare studies. Therefore, a round table conference in 2006 convened to discuss the various aspects of image acquisition and the different scores, and a consensus statement was drafted (24).The scores that can be used to describe numerically the microcirculatory images consist of the following: a measure of vessel density (total and perfused vessel density); two indices of perfusion of the vessels (proportion of perfused vessels and microcirculatory flow index); and a heterogeneity index. In addition, this information should be provided for all vessels and for small vessels (mostly capillaries) identified as smaller than 20 μm (24).

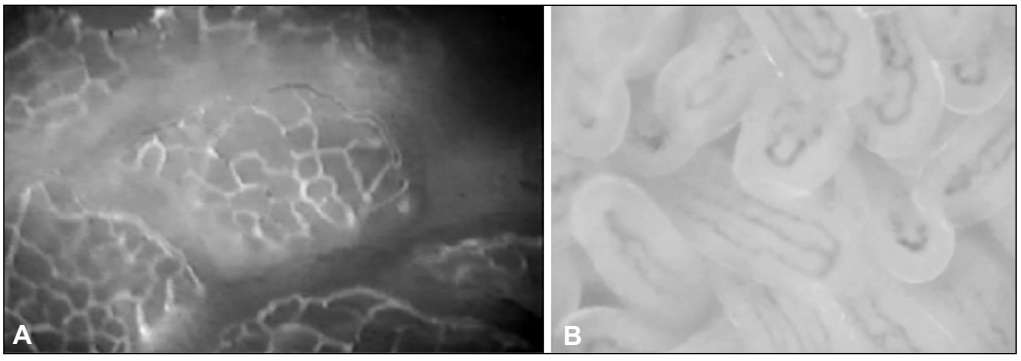

Figure 1: ***A:*** *Intestinal microcirculation of rat mucosa observed by fluorescence (FITC albumin) intravital microscopy. All capillaries are well perfused indicated by bright plasma contrast.* ***B:*** *Intestinal microcirculation of human ileostomy – mucosa observed by SDF monitoring. Note: Perfused capillaries are dark representing scattered light by red blood cell hemoglobin.*

Table 1: Clinical studies of the intestinal microcirculation using OPS or SDF imaging

Device	Target	Results	Reference
OPS	IMIC vs. sublingual	dispersion on day 1 of sepsis	(28)
OPS	IMIC (rectum)	extensive microvascular obstruction in severe falciparum malaria	(26)
SDF	IMIC (rectum)	PPV <90% in 54% of all patients after elective CABG	(25)

OPS – orthogonal polarization spectral imaging, SDF – sidestream dark-field imaging, PPV – proportion of perfused vessels, CABG – coronary artery bypass grafting

Clinical studies using imaging of the intestinal microcirculation

Since imaging of the intestinal microcirculation is not easy to obtain in the clinical routine, only a few clinical studies are available employing microcirculatory diagnostics and targets (Table 1). Two studies used the rectal mucosa for studies of the intestinal microcirculation. One study compared the sublingual microcirculation and the IMIC assessed via enterostomies.

The Dutch group of Boerma at al. published a study in patients after elective cardiac surgery using SDF technology (25). Postoperatively, direct *in vivo* observation of rectal mucosa revealed a PPV $<90\%$ in 54% of all patients. At the same time, rectal microcirculatory blood flow appeared to be unaltered. Combining rectal SDF imaging with rectal tonometry revealed a 7% incidence of rectal-to-arterial $pCO2$ gap >1.4 kPa, suggesting non-dysoxic perfusion in the majority of patients, despite the observed percentage of non-perfused crypts.

Dondorp et al. sought to describe and quantify microcirculatory changes in the rectal mucosa of patients with severe malaria, by direct in vivo observation using OPS imaging (26). Patients with severe falciparum malaria showed extensive microvascular obstruction that was proportional to the severity of the disease. This finding underscored the prominent role that microvascular obstruction plays in the pathophysiology of severe malaria and illustrates the fundamental difference between the microvascular pathophysiology of malaria and that of bacterial sepsis.

A study of the relationship between sublingual and intestinal microcirculatory perfusion in patients with abdominal sepsis showed that on day 1 of abdominal sepsis there is a complete dispersion of flow, not only between hemodynamic compartments of a different order but also between the sublingual and intestinal microcirculation. Over time, both sublingual and intestinal microvascular flow indexes trended to normal values (28).

Future developments

Recent technological advances in clinical microcirculatory image acquisition and analysis now permit microcirculation-targeted treatment of intestinal pathologies by providing instant feedback on the efficacy of the applied therapeutic strategies at the microcirculatory level. However, at present the usability of the available devices is still limited. The routine use is restricted to well-trained specialists and patients with enterostomy (or intra-operatively). To facilitate future developments in intestinal microcirculation research, further improvements of the hard- and software are required. Regarding the imaging devices further miniaturization of the optical probes could reduce pressure artifacts and improve reproducibility of the measurements. Endoscopic applications or

swallow-able cameras should be developed. A major contributor to interobserver variability is also the manual part of the software analysis. Further automatization of the measurements is under development (27).

In conclusion, it is evident that microcirculatory dysfunction is important in different intestinal pathologies. Therefore, microcirculatory diagnostics and targets should be integrated in the therapeutic strategies in intestinal diseases.

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