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Comparison of the New OPS Imaging Technique with Intravital Microscopy: Analysis of the Colon Microcirculation

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Key Words
Microcirculation · Intravital microscopy · Intestinal · OPS imaging · Inflammatory bowel disease

Abstract
Background: The OPS imaging technique has been introduced for in vivo assessment of microcirculation in humans. The aim of this study was to validate the new technique against intravital fluorescence microscopy (IFM) for the visualization of colon microcirculation in a murine model of inflammatory bowel disease (IBD). ***Method:*** IBD was induced in Balb/c mice by dextran sulfate sodium, controls received normal water. In each animal, both the CYTOSCAN™ A/R and IFM were used to image the microcirculation (n = 7 in each group). The postcapillary venular diameter was analyzed on the colon muscularis and mucosa. ***Results:*** The venular diameter correlated significantly between both methods representing the good correspondence between both methods. ***Conclusion:*** Our study demonstrates that the new technique for visualization of microcirculation without use of fluorescent dyes, the OPS imaging, allows for quantitative measurement of a key microcirculatory parameters of the mouse colon.

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Introduction

The microcirculation is the link between blood and tissue. Investigations of the microcirculation have contributed to a more detailed understanding of the pathophysiological changes which occur in disease and injury. These studies have been carried out in numerous animal models using intravital fluorescence microscopy. However, this method requires systemic application of potentially toxic [1–3] fluorescent dyes (e.g. rhodamine-6G, fluorescein-isothiocyanate) and large, expensive instrumentation (intravital microscopy). Because of these limitations, investigations of the microcirculation in humans have been restricted to the skin, nail fold and conjunctiva [4, 5].

Recently, a new microscopic technique, orthogonal polarization spectral imaging (OPS imaging), implemented in a portable device, the CYTOSCAN™ A/R (Cytometrics, Inc., Philadelphia, Pa., USA), has been introduced [6] as a novel method for the visualization of the microcirculation. This method uses polarized reflected light instead of fluorescence light and allows for imaging of the microvasculature noninvasively through mucus membranes and on the surface of solid organs. When microcirculatory data obtained using this novel method were compared with data obtained by conventional intravital fluorescent microscopy (IFM) a significant correlation was found between both methods in the striated muscle in the hamster dorsal skinfold chamber and the rat liver [7, 8].

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The investigation of the intestinal microcirculation and its changes in animal models of inflammatory bowel diseases (IBD) using IFM has allowed for in vivo assessment of the extent of the lesion, the status of inflammation and the effect of potentially new therapeutic approaches [9–11]. However, because of the limitations mentioned previously microcirculatory studies have been restricted to animal models.

The analysis of human colon microcirculation would be of great diagnostic value in inflammatory disease and bowel resection [12]. With the CYTOSCAN A/R for the first time a device is available which may allow the investigation of human colon microcirculation for in vivo assessment of microvascular perfusion alterations of the gut. With this device it should be possible to examine the colon microcirculation on the abluminal wall of the gut (the vessels of the muscularis) within a surgical setting or from the luminal (mucosa) within an endoscopic setting. To prepare for this application in the clinic the aim of our study was to validate OPS imaging against conventional IFM for the quantitative measurement of microvascular parameters in the colon microcirculation. The study was performed in the mouse and microcirculatory measurements were carried out using both the CYTOSCAN A/R and conventional IFM on the abluminal and luminal wall (muscularis and mucosa) of the colon under control conditions and after induction of IBD. This article is based on a publication submitted to the *European Journal of Medical Research* [23].

Material and Methods

Induction of Inflammatory Bowel Disease

For the validation under pathophysiological conditions a chronic IBD model in the mouse was chosen. Fourteen female Balb/c mice at the same age of 4 weeks were divided into a colitis and a control group. Rodents were kept under standard laboratory conditions and allowed free access to animal chow and tap water. IBD was induced according to Okayasu et al. [13] by adding 5% dextran sulfate sodium to the drinking water provided ad libitum. The colitis group received Dextran sulfate sodium (DSS)-containing water over 7 days followed by normal drinking water for 10 days comprising a complete cycle of 17 days. After 3 of such cycles, e.g. 51 days the animals were examined. The control group received normal drinking water over the same period of time. The animals were placed on a water diet 12 h prior to surgery. The experiments were performed in accordance with the German law for the protection of animals.

Surgical Procedure

The mice were anaesthetized through inhalation of isoflurane-N_2O and placed in supine position on a heating pad for maintenance of body temperature between 36 and 37 °C. Polyethylene catheters were inserted into the left carotid artery and jugular vein for recording of mean arterial pressure, blood sampling, volume replacement of 10 ml $kg^{-1} \cdot h^{-1}$ Ringer's lactate and for the injection of fluorescent dye [14]. The surgical procedure was performed as described previously [9]. As previously described [15], the coordinates of the different regions of interest (ROIs) were stored in the computer and could be relocated at later time points during the experimental protocol.

Microscopic Measurements

IFM was performed as previously described by our group through standardized scanning of 6–8 nonoverlapping ROI over a period of 20 s each [14]. The microscopic images were captured using a CCD video camera (FK 6990, Cohu, Prospective measurements, San Diego, Calif., USA) and recorded on S-VHS video tape using a sony video recorder (Sony, Munich, Germany) for off-line evaluation. The plasma marker, fluorescein-isothiocyanate (FITC)-labeled dextran (0.05 ml, 5%; mol wt 150,000, Sigma, St Louis, Mo., USA) was injected intravenously for contrast enhancement for the IFM observations [14].

OPS imaging was performed using the CYTOSCAN A/R device in the identical 6–8 nonoverlapping ROIs [for technical details of CYTOSCAN A/R, see 6]. The CYTOSCAN A/R images were captured using a CCD videocamera and recorded with the same videorecorder for off-line evaluation. With a ×10 objective and a video screen (PVM-1442 QM, diagonal 33 cm, Sony, Munich, Germany) a magnification of ×450 was reached.

After the images of the muscularis were recorded, the gut was incised longitudinally (approximately 10 mm) in an antimesenterical direction with a microcauter [14]. Because the mucosa is a very sensitive tissue all procedures were performed very gently. The space available for investigation of microcirculation (app. 1 cm^2) was smaller than in the muscularis, therefore the number of ROIs had to be reduced to 4–5 in the mucosa. The microcirculation was examined in the same manner using first the IFM and then the CYTOSCAN A/R. To avoid alterations of microcirculation due to the examination itself the surface of mucosa was exposed only to Ringer's lactate solution needed for visualization of microcirculation by means of water immersion objective and for keeping the gut moist.

After finishing the recording of images the animals were sacrificed by an overdose of pentobarbital.

Image Analysis

Quantitative assessment of microcirculatory parameters was performed off-line from the video-taped images in a 'blinded' manner concerning the presence of colitis. The following microcirculatory parameters were assessed: venular diameter, venular red blood cell velocity (RBC-V) and functional capillary density (FCD). The diameter of postcapillary venules was assessed using the manual function (DiaMa) of CapImage® (Dr. H. Zeintl, Heidelberg, Germany) [16]. Vessels with a diameter between 20 and 80 μm were considered to be postcapillary venules.

Histological Examinations

To verify induction of IBD, tissue specimes from the segment studied were taken immediately after sacrifying the animal. Three paraffin interval sections of the longitudinal colon with a distance of 30 μm per specimen were performed and stained with H and E. The severity of colitis of each slice was graded on a scale from 0 to 3 using the index introduced by Onderdonk et al. [17]: 0, normal; 1, focal inflammatory cell infiltration, gland dropout, and crypt abscess; 3, mucosal ulceration. The scores of the single slices were sampled

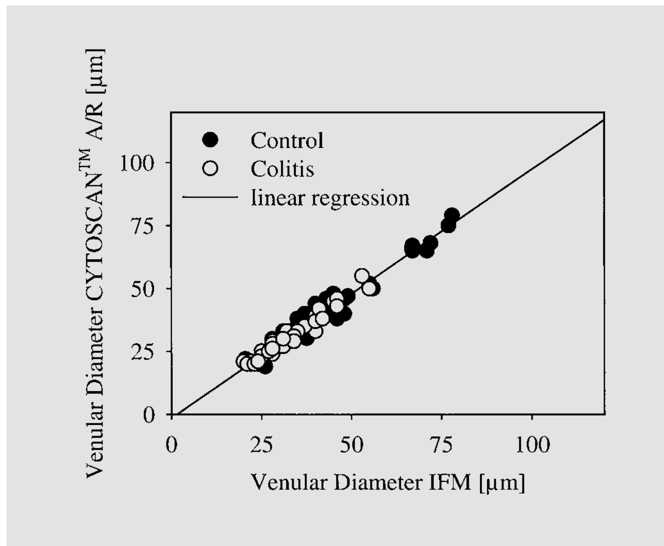

Fig. 1. A combined linear regression analysis of measurements of the postcapillary venular diameter on the muscularis of control and colitis group obtained from IFM plotted on the x-axis and those from CYTOSCAN A/R on the y-axis.

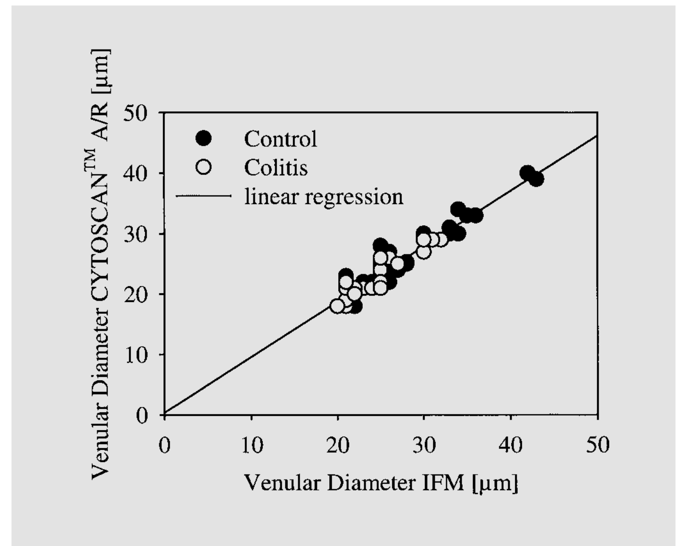

Fig. 2. A combined regression analysis of the measurement on the mucosa with values obtained from IFM printed on the x-axis and those from CYTOSCAN A/R on the y-axis. Again, the slope of the linear fit is close to 1 and the data accumulate very narrow around the linear fit indicating an excellent agreement of both methods. Spearman's correlation coefficient was significant at $p < 0.05$.

(3 per animal) and a mean value calculated for each animal which was used for statistical analysis.

Statistics

The relationship between values obtained using IFM and the CYTOSCAN A/R were analyzed by linear regression and Spearman rank order correlation coefficient. Differences between the control and colitis groups were analyzed using Mann-Whitney U test. Data analysis was performed with statistical software package (SigmaStat for Windows, Jandel Scientific, Erkath, Germany) and all values are given as mean ± SD. Significance was set at p values < 0.05.

Results

A total of 6–8 postcapillary venules/capillary networks in the muscularis and 4–5 postcapillary venules/capillary networks in the mucosa were scanned per animal in altogether 14 mice.

The morphological structures of the intestinal microcirculation of both layers, muscularis and mucosa, could be clearly identified with both CYTOSCAN A/R and IFM.

Comparison of Microcirculatory Parameters

Venular Diameter. The results from measurement of postcapillary venular diameter (control and colitis) in the muscularis revealed an excellent correlation ($y = 0.99x - 1.47$, $r^2 = 0.97$, standard error of estimate 3.11, n = 92, $p < 0.05$) between OPS imaging and IFM (fig. 1). The regression analysis of values of the venular diameter in the mucosa is shown in figure 2 and also indicates a very good correlation between both methods ($y = 0.92x + 0.40$, $r^2 = 0.91$, standard error of estimate 1.69, n = 66, $p < 0.05$).

Comparison between Control and Colitis Group

The histological examination of specimens obtained from the control group revealed a mostly normal colonic mucosa, whereas the colitis group showed typical pathological characteristics of chronic IBD, i.e. crypt abscess and mucosal ulceration. A mean 'colitis score' of 0.64 ± 0.14 points was found in the control group versus 2.31 ± 0.20 points of the colitis group ($p < 0.05$).

A synopsis of the data obtained from control versus colitis animals is given in table 1 for the muscularis and the mucosa. The measurements of the postcapillary venular diameter was significantly different between both groups. These differences were present in the muscularis as well as in the mucosa.

Table 1. Microcirculatory parameters obtained from OPS imaging and IFM from control and IBD groups

	Control		Colitis	
	IFM	CYTOSCAN A/R	IFM	CYTOSCAN A/R
Venular diameter, μm				
Muscularis	42 ± 6	40 ± 7	32 ± 3*	30 ± 3*
Mucosa	28 ± 3	26 ± 2	25 ± 1*	23 ± 1*

Data are expressed as means ± SD.
* $p < 0.05$ in Mann-Whitney U test, control vs. colitis.

Discussion

We hereby clearly demonstrated that the new technique, OPS imaging, implemented into the CYTOSCAN A/R allows for quantitative assessment of the intestinal microcirculation in the normal and inflamed gut to determine the parameter postcapillary venular diameter. Hence, the quantitative measurement of microvascular parameters in the murine colon using OPS imaging against conventional IFM has been validated. Our results indicate that when IFM technique allows accurate and precise measurements, OPS imaging does also (venular diameter).

The obtained pictures of the colon microcirculation using IFM and OPS imaging in our investigation corresponded very closely to what is known as normal appearance of the microvessels in the intestinal muscularis and the mucosa [14, 18]. With the OPS imaging technique it was possible to obtain high-quality images of the microcirculation of the murine colon without fluorescent dye. From these images accurate quantitative measurements of the postcapillary venular diameters could be made.

Venular Diameter

In our study, the measurement of venular diameter in the muscularis and mucosa (table 1) revealed a systematic bias of approximately 2 μm when one compares values obtained by IFM and CYTOSCAN A/R. The cause for this significant bias is probably due to the fact that OPS images the hemoglobin carrying structures, e.g. red cells, red cell column, while IFM with FITC-dextran pictures the plasma, containing spaces. It is known that at physiological conditions the endothelial layer of the capillaries is covered by a layer of surface proteins of approximately 1 μm width hindering erythrocytes to contact directly the vessel wall [19]. In contrast, the molecules of the FITC-dextran used for IFM are smaller than red blood cells and therefore enter the plasma layer on the endothelial surface. Hence, measurement of venular diameter by means of IFM using FITC-dextran as a fluorescent dye comprises the endothelial plasma layer and therefore yields an overestimation of the internal diameter of approximately 2 μm as compared to CYTOSCAN A/R. Thus, the systematic bias observed in this study was to be expected. However, despite this systemic bias in our study the values of diameter measurements obtained by the established IFM and the new CYTOSCAN A/R correlated significantly to each other which supports experimental data on measurements of diameters obtained in the dorsal skinfold chamber model of the hamster [7] and in the liver [8] as well as most recent data of diameters of the nailfold capillaries in patients by Mathura et al. [18].

Comparison of Control vs. Colitis Group

The histological appearance of the chronic colitis and the quantitative colitis score in our study was in line with previously published data using the DSS model [17, 20, 21]. Hence it can be concluded, that our model should allow for investigation of the colon microcirculation under control conditions and after induction of IBD.

The postcapillary venular diameters was significantly reduced in IBD animals compared to healthy controls. In this context, in vitro investigations using microradiography on bowel specimens removed from patients suffering from IBD demonstrated a significantly reduced vascularity of the colon submucosa in the parts affected by segmental Crohn's disease [22].

Conclusion

Our study demonstrated that the CYTOSCAN A/R allows the visualization of the microcirculation of the mouse colon under physiological and pathophysiological conditions. The quantitative analysis from the obtained images showed a significant correlation of the data obtained by using the CYTOSCAN A/R compared with those by using IFM.

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