Int J Colorect Dis (1999) 14: 29–34

ORIGINAL ARTICLE

T. Foitzik · M. Kruschewski · A. Kroesen · H. J. Buhr

Does microcirculation play a role in the pathogenesis of inflammatory bowel diseases?

Answers from intravital microscopic studies in animal models

Accepted: 23 December 1998

Abstract The potential role of intestinal microcirculation for the development of inflammatory bowel diseases (IBD) has not been systematically investigated, mainly because of methodological problems. Using a well-established rodent model of IBD and intravital microscopy, the present study investigated whether (and when) gut microcirculation is disturbed in IBD, and whether microcirculatory disorders contribute to histological and functional alterations in the development of IBD. Colitis was induced by rectal injection of trinitrobenzene sulfonic acid. After 1, 3, and 15 days rats were laparotomized for intravital microscopic determination of mucosal colonic blood flow. In a second series it was examined whether enhancing colonic capillary blood flow by hemodilution therapy stabilizes colonic wall resistance and other electrophysiological parameters of gut permeability. Additional measurements involved hemodynamic monitoring and histological examinations. Colonic capillary blood flow was significantly decreased 3 days after colitis induction (1.8 ± 0.05 vs. 2.6 ± 0.04 nl/min in healthy control animals) when histology revealed signs of acute inflammation, and normal values after 15 days (2.4 ± 0.06 nl/min) when chronic histological changes were evident. Hemodilution therapy enhanced colonic capillary blood flow in the initial stage (2.1 ± 0.02 vs. 1.6 ± 0.02 nl/min in saline-treated animals with trinitrobenzene sulfonic acid colitis) and improved gut resistance and electronic chlorid secretion (73 ± 15 vs. 33 ± 8 μA cm^2). Histological alterations were not significantly attenuated. Impaired colonic capillary blood flow in the initial stage of experimental colitis and improved mucosal microcirculation with stabilized gut permeability suggests that the early microcirculatory disturbances precede chronic histological changes and influence functional alterations in the course of the disease. Research should be continued in this field because important mechanisms in the pathogenesis of IBD and potentially therapeutic (vasoactive) substances may otherwise be overlooked.

Key words Inflammatory bowel disease · Animal model · Microcirculation · Intravital microscopy · Intestinal barrier · Gut permeability

T. Foitzik (✉) · M. Kruschewski · A. Kroesen · H. J. Buhr
Department of Surgery, Benjamin Franklin Medical Center, Freie Universität Berlin,
Hindenburgdamm, D-12200 Berlin, Germany
e-mail: foitzik@ukbf.fu-berlin.de,
Fax: +49-30-84452740

Introduction

No single agent or mechanism explains the pathogenesis of inflammatory bowel diseases (IBD). It has become clear that many determinants are involved from predisposing genetic and environmental factors to noxae such as microbial agents which may trigger the onset and promote the disease [1]. In the past few years immune factors have been of central interest in mediating the inflammatory reactions, whereas the potential role of nonimmune cells has virtually been neglected in the pathogenesis of IBD. This is surprising since electron microscopic studies have demonstrated cell damage of the entire intestinal wall including the vascular epithelium early in the diseases process [2–9].

Endothelial cells are not only the "gatekeepers" for the extravasation of circulating leukocytes and inflammatory cytokines into the interstitium, but an intact endothelium is also essential for undisturbed capillary blood flow which is vital for transporting oxygen and nutrients and therefore necessary to maintain normal mucosal homeostasis, intestinal permeability, and gut barrier function. Despite these important functions (previously recognized in many other diseases), intestinal microcirculation has not been systematically investigated in IBD, and no answers have been found to the question of whether gut endothelium and changes in the intestinal microvasculature and microcirculation contribute to the pathogenesis of IBD.

The potential role of vascular factors in IBD has not been properly addressed in the past mainly because of methodological problems. Only a few groups have established adequate experimental techniques for measuring changes

in the capillary bed of the bowel in vivo, and there is no method for visualizing intestinal microcirculation in humans. Moreover, the primary focus of the few experts in the field of microcirculation is not IBD, which has recently been dominated by gastroenterologists working on genetic and molecular-biological topics.

Based on our experience with intravital microscopy of the pancreas, liver, lung, and colon in other animals models [10–12] we are now investigating microcirculation in experimental IBD with the aim of answering the following questions: (a) Is gut microcirculation disturbed in IBD, and, if so, at what point in the development of IBD does it become evident? (b) Do microcirculatory disorders contribute to the development of IBD and disease severity?

This contribution summarizes the findings from a series of animal experiments performed in our microcirculation laboratory. Our results show significantly impaired colonic capillary blood flow in the first few days (acute stage) of experimental IBD, suggesting that microcirculatory disturbances precede (chronic) histological changes. Moreover, we found that improved colonic capillary blood flow is associated with enhanced gut barrier function. This strongly indicates that disturbed gut microcirculation plays a role in the evolution of IBD, and that research should be continued in this field because important mechanisms in the pathogenesis of IBD and potentially therapeutic (vasoactive) substances may otherwise be overlooked.

Methods

All experiments were conducted in accordance with the national guidelines for the use and the care of laboratory animals and approved by the local ethics committee.

Colitis induction

After 24 h of fasting, male Sprague-Dawley rats (330 ± 20 g) were subjected to IBD induction as described by Morris et al. [13]. Under light ether anesthesia animals were placed in supine position and given a single perianal infusion of 0.25 ml trinitrobenzene sulfonic acid (TNBS) acid via a soft polyethylene catheter which was introduced 5 cm from the anus into the descending colon and kept in place for 15 min after the injection. Two groups of control animals received a rectal infusion of either water or ethanol.

Intravital microscopy of the colonic mucosa and quantification of colonic capillary blood flow

At different time points after colitis induction (see "Experimental design") animals were reanesthetized with an intramuscular injection of ketamine (40 mg/kg) and pentobarbital (20 mg/kg), equipped with an intra-arterial and intravenous polyethlyene catheter (ID 0.5 mm) inserted into the carotid artery and jugular vein, and placed on a heated operating table to maintain body temperature at 37 ± 1 °C. The abdomen was opened through a lower midline incision and the colon exteriorized and fixed with sutures on a plexiglass stage and continuously moistened with Ringer's solution of 37 ± 1 °C. Special care was taken not to comprise perfusion by putting tension on the mesentery. After determining subserosal (muscular) colonic capillary perfusion (data not shown), the colon was opened at the antimesenteric border with microscissors. Special care was taken to minimize bleeding and to avoid mechanical damage to the mucosa. Subsequently, capillary perfusion of the colonic mucosa was assessed contralateral to the incision line by intravital microscopy (IVM). IVM and colonic capillary blood flow (CCBF) determination were performed as previously described elsewhere [11].

Briefly, animals received an intravenous injection of homologous erythrocytes (hematocrit 50%) labeled with fluorescein isothiocyanate (0.2 ml/kg FITC Isomer I, Sigma; Deisenhofen, Germany). Thereafter they were placed under a fluorescence microscope (Leitz; Wetzlar, Germany) and allowed to stabilize for 10–20 min. Epi-illumination was achieved with an arc xenon lamp (XBO 100 W/2; Osram, Berlin, Germany) in the presence of a heat-protecting and excitation (450–490 nm) filter. Ten randomly chosen regions (each 400×325 µm) of the mucosa were recorded in each exposed colon segment. The microscopic picture was transferred to a monitor via video camera (Cohu CCD-4810; San Diego, Calif., USA) and recorded on videotapes for subsequent off-line analysis using a computer-assisted image analysis system (Cap Image [14]). Off-line analysis of video-recordings was performed in a blinded manner by an independent observer (H.H). For each recorded region CCBF was calculated in an average of 20 capillaries by correlating the number of passing labeled erythrocytes to the capillary hematocrit. The concentration of fluorescent erythrocytes per unit of arterial blood was measured in all animals by counting the cells in 50 different fields of a Neubauer Chamber. Capillary hematocrit was calculated by multiplying systemic hematocrit by 0.76, a factor previously described as a constant capillary-to-systemic hematocrit ratio for capillaries of rodents of similar size [15].

Hemodynamic monitoring and exclusion criteria

During IVM, mean arterial pressure (MAP) was monitored continuously using an electronic sphygomanometer. Arterial blood gases were also repeatedly measured from blood samples. Since deterioration of systemic cardiorespiratory function may compromise the interpretation of microcirculatory changes, animals were excluded when one of the following criteria was present: MAP < 80, pCO_2 > 50, pO_2 < 80 pH < 7.3 or > 7.5 at any time point during IVM. Any kind of surgical trauma to the preparation was another exclusion criteria.

Electrophysiological determination of colonic permeability

To answer the second question (see "Experimental design"), electrophysiological determination of colonic permeability was performed in a modified Ussing chamber as described previously [16, 17]. Briefly, a colon segment adjacent to that used for IVM was harvested, washed in iced medium to remove luminal content, and partially stripped. The serosal side of the tissue was glued with Histoacryl (Braun, Melsungen, Germany) to a plastic ring (ID 9 mm) and inserted between the two halves of the chamber. The effective chamber area was 0.28 cm^2. Bathing fluid consisted of (mM) Na^+ 140.5, K^+ 5.4, Ca^{2+} 1.2, Mg^{2+} 1.2, Cl^- 123.8, HCO^{3-} 21, HPO^{4-} 0.6, D(+)-glucose 10, β-OH-butyrate 0.5, glutamine 2.5, D(+)-mannose 10. A combination of 50 mg/l azlocillin (Securopen, Bayer, Leverkusen, Germany) and 10 mg/l imipenem (Zienam, MSD Sharp & Dohme, Munich, Germany) were added to avoid bacterial overgrowth in the course of the experiment. Solutions were gased with 95% O_2 and 5% CO_2 and had a pH of 7.4. All Ussing chamber measurements were performed at a constant temperature of 37 °C. Unidirectional ^{3}H-labeled mannitol flux studies from mucosa to serosa were performed under short circuit conditions. Mannitol flux as a parameter of colonic permeability was calculated using a standard formula. In addition, we measured epithelial (mucosal) and subepithelial resistance and electronic Cl^- secretion after stimulation with theophylline and prostaglandin (PG) E2 (ΔI_{SC}).

Histology

At the end of the experiments colon segments adjacent to those used for IVM or electrophysiological examination were harvested for his-

tological examination. The tissue samples were fixed in neutral buffered 4% formalin and routinely processed before embedding in paraffin. Eight transverse slices of 4–5 μm were taken, stained with hematoxylin-eosin, and subjected to light microscopy performed in a blinded manner by a pathologist with experience in IBD (P.C.) in a blinded manner. A histological score ranging from 0 to 20 [18] was used to quantify the extent of both acute and chronic inflammation in the colonic wall.

Statistics

Data are represented as means ± SEM. Continuous variables were tested for group differences using Student's unpaired *t* test or analysis of variance when appropriate. A 5% probability of type I experimental error ($P < 0.05$) was accepted as statistically significant.

Experimental design

Question 1

To answer the question of whether (and if so when) there are microcirculatory disturbances of the gut in IBD, colonic capillary blood flow was determined in eight to ten animals at different time points after colitis induction. According to previous observations [18], IVM was performed 1, 3, and 15 days after colitis induction. Two areas were prepared for IVM: one in a macroscopically inflammed segment of the descending colon and on in a macroscopically intact segment of ascending colon. Also, two to four healthy control animals were observed at each time point.

Question 2

To answer the second question, we hypothesized that if microcirculatory disturbances contribute to the development of IBD and disease severity, improving microcirculation will ameloriate disease sequelae. To improve colonic microcirculation we used hemodilution therapy with low molecular weight dextran (70 kDa; Pharmacia, Erlangen, Germany) which has a well-described beneficial effect in macro- and microcirculation but no known immunological one [19, 20]. As a parameter of disease severity, we used ion permeability of the gut previously demonstrated to be increased in both TNBS colitis in rats [21] and patients with ulcerative colitis [22]. Since these changes have been observed early in the disease course, the following protocol was chosen: TNBS colitis was induced in 16 rats randomized for either hemodilution therapy with dextran 8 ml/kg dextran 70,000 or an infusion of 8 ml/kg normal saline. Therapy was started 12 h after colitis induction. After 48 h all rats underwent laparotomy, and the descending colon was prepared for IVM. Thereafter a colon segment adjacent to that used for IVM was excised, partially stripped, and mounted in the Ussing chamber for electrophysiological examination as described above. Another segment was used for histological examination.

Results

Drop outs

According to the above criteria 1 of 18 control animals and 6 of 44 animals with colitis had to be excluded.

Healthy control animals

Determination of colonic capillary blood flow in the exposed mucosa revealed values between 2.4 and 2.8 nl/cap per minute with no significant differences between observation sites (ascending or descending colon) or the time point after the sham procedure (rectal infusion of ethanol or water). Histology showed no or only minor signs of acute tissue injury (median score: 0 points; score range 0–1 points).

Table 1 Mucosal capillary blood flow (*CBF*, nl/min) and histological score (points) in the ascending and descending colon at various time points after colitis induction

Group	Time after induction	Ascending colon		Descending colon	
		CBF	Histology	CBF	Histology
Control	(varia)	2.5 ± 0.04	0–1	2.6 ± 0.04	0–1
Colits					
1 day		2.5 ± 0.05	0–1	2.9 ± 0.05	7–12
3 days		2.4 ± 0.06	0–1	1.8 ± 0.05 *	4–12
15 days		2.4 ± 0.08	0–3	2.4 ± 0.06	1–11

* $P < 0.05$ vs. healthy control animals and other time points

TNBS colitis at different time points (Table 1)

In the descending colon with TNBS colitis, edematous swelling and hyperemia were seen macroscopically on the first day and ulcerations and confluent cell death on the third day, which was replaced by scar tissue after 2 weeks. Severe acute inflammation was initially found in the histological examination. Granulocytic infiltration and ulceration of the mucosa were especially pronounced here and severer in the first 3 days than on the 15th day. The ascending colon was macroscopically normal, but round cell infiltration was determined histologically after 2 weeks as a sign of mild chronic inflammation.

Mucosal capillary blood flow in the descending colon was significantly higher than that in healthy controls on the first day after induction and significantly lower after 3 days. Normal values were measured after 15 days. Perfusion in the ascending colon did not significantly differ from that in healthy animals.

TNBS colitis with hemodilution therapy (Table 2)

In agreement with the results of the first test series, capillary perfusion in the mucosa of the descending colon was significantly lower than in healthy animals 2 days after induction of TNBS colitis (saline group). Hemodilution with dextran significantly improved CCBF.

Saline-treated animals with TNBS colitis had a significantly lower subepithelial (mucosal) resistance in the descending colon, more mannitol flux, and lower active transport capability (Cl^- secretion) than healthy controls (with rectal infusion of ethanol or water). Hemodilution therapy with dextran improved all electrophysiological parameters. Due to the small number of animals ($n = 7$) and a relatively large standard deviation, differences to saline-

Table 2 Mucosal capillary blood flow (*CBF*) and electrophysiological parameters of gut permeability of the descending colon 2 days after colitis induction and hermodilution therapy or saline infusion. (R_e Epithelial resistance, R_s subepithelial resistance, ΔI_{SC} electronic Cl^- secretion)

	Colitis + saline	Colitis + hemodilution
CBF (nl/min)	1.6 ± 0.02	2.1 ± 0.02*
R_e (Ω cm^2)	17.9 ± 2.8	20.2 ± 5.2
R_s (Ω cm^2)	4.5 ± 1.4	6.0 ± 1.3
ΔI_{SC} (μA cm^2)	33 ± 8	73 ± 15*

* $P < 0.05$

treated animals, however, were statistically significant only for total bowel wall resistance and the active ion transport capability (Cl^- secretion).

The histological changes were not significant attenuated by therapy given after a 10-h delay.

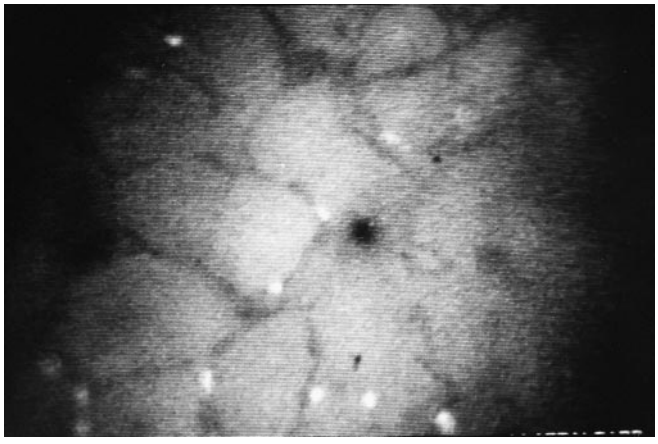

Fig. 1 Capillary network of the colonic mucosa with fluorescein-labeled erythrocytes

Discussion

The first question of our study, "Is gut microcirculation disturbed in IBD, and, if so, at what point in the development of IBD does this become evident?" cannot be answered by clinical studies since it is not possible to measure changes in human gut microcirculation in vivo. However, there is indirect evidence for endothelial lesions and instestinal capillary perfusion disorders in Crohn's disease and ulcerative colitis even in the early stage of the disease. Electron microscopic examination reveals arteritislike vascular changes and thrombotic vessel occlusion in the subserosa and submucosa [3]. Moreover, a significant reduction in mucosal perfusion in the rectum of patient with ulcerative colitis has been demonstrated by laser Doppler flowmetry [23]. This also agrees with spectroscopic findings indicating decreased perfusion in the area of fresh ulcerations in experimental colitis [24]. Signs of greater leukocyte-endothelium interaction have been determined in both the histological and intravital microscopic examinations [3, 24], which reflects the increased adhesion molecule expression found in IBD patients [25]. It is exactly these changes which are considered of great importance today since they not only promote "homing," i.e., the infiltration of circulating lymphocytes into the bowel wall and thus foster perivascular inflammatory reactions, but also lead to plasma loss from the intravasal space and interstitial edematous swelling. In this way oxygen and nutrient transportation is impaired, and blood viscosity increased by intravasal fluid loss, which in turn restricts capillary blood flow and thus the supply of enterocytes and colon cells.

Although intestinal wall edema and intravasal fluid loss (decreased central venous pressure, increased hematocrit) are also seen in IBD patients, the above observations only indirectly confirm the presence of microcirculatory disorders of the intestinal wall in Crohn's disease and ulcerative colitis. Thus, the aim is to directly visualize capillary changes in the mucosa. Although IVM is the methodological gold standard for examining microcirculation, it cannot be applied clinically except in a few areas such as the nail bed groove. There is controversy over the value of alternative methods for visualizing capillary changes. These methods include microsphere techniques, laser Doppler flowmetry, hydrogen clearance, and various spectroscopic procedures. Data from spectroscopic measurements has been the most extensively evaluated, for example, in plastic surgery for monitoring flap perfusion [26] and Japanese studies (via endoscope) for measuring perfusion in the abdomen and duodenum [27, 28]. Individual clinical data using this method in the intestine suggest an increase in mucosal blood flow in IBD patients but are inconclusive and have not been reproduced [29, 30].

Against this background, the primary aim of our study was to measure perfusion in the intestinal mucosa in an animal model of IBD. This step seemed useful since a great deal of knowledge about the pathogenesis of IBD has been gained from animal models, and some of these models are being used for preclinical testing of new IBD drugs. For reasons of practicability (e.g., performing tests in rats, inexpensive procurement of agents for colitis induction, good model characterization), we chose TNBS colitis, which has been widely used in recent years and is recognized as a standard model of experimental IBD [31]. While IVM in the liver or pancreas of rats has been an established procedure for many years, only very few groups have thus far succeeded in generating assessable images of microcirculation in bowel mucosa. Although Leung and Koo [32] described marked vascular stasis and increased capillary permeability in the mucosa of rats with acetic acid induced colitis, they were not able to quantitatively these changes due to methodological limitations. Our own images (Fig. 1) and data obtained with state-of-the-art equipment and improved techniques confirm (a) that there are microcirculatory changes in TNBS colitis, and (b) that these changes already occur in the acute inflammatory and not in the chronic stage.

In the TNBS model an extensive unspecific acute inflammatory reaction is demonstrated in the first few days,

which is also reflected by hyperemia, i.e., an increase in capilllary blood flow. Surprisingly, there is a marked decrease in capillary blood flow after 3 days, although further acute inflammatory signs are still present histologically. Changes such as granulomas that are typical for this model are seen only at a later time point, when mucosal capillary perfusion has normalized. Based on this finding, we believe that disturbed microcirculation precedes the specific morphological changes in the mucosa and may thus be involved in functional disorders responsible for disease severity in the acute stage and contributes to disturbances that cause (septic) complications in the later course.

Our observations indicate that microcirculatory disorders occur so early in experimental colitis that they cannot be a sequela of inflammation; however, they do not provide any insights into the role of microcirculatory disorders in the pathogenesis of IBD. The question of whether microcirculatory disorders contribute to the development of IBD and disease severity cannot be directly answered in any case. If microcirculatory disorders are of pathogenic importance, their improvement in the early phase of colitis must ameliorate the course of the disease. The hypothesis that improved microcirculation attenuates the severity of TNBS colitis was investigated in the second trial by applying a colloid plasma expander, which had a positive influence especially on microcirculation but no immunological effect [19, 20]. Much more problematic than ameliorating microcirculation was the choice of a suitable parameter for verifying the improvement in TNBS colitis. Considering the absence of mortality or clinic parameters like stool frequency, electrolyte shifting, blood count alterations or fever, it seemed appropriate to take morphological changes as the criterion of disease severity. On the other hand, histological changes do not reflect disease severity nor are they correlated with relevant functional changes (e.g., diarrhea) [33, 34]. Moreover, typical histological changes occur only at a later time point in the TNBS model, when colonic capillary blood flow has already normalized, and histological changes have a relatively larger fluctuation range which can be recorded only semiquantitatively with standardized scores. Using human colitis for orientation we selected intestinal permeability as the functional parameter of disease severity. This seems justified since human colitis in the acute stage is characterized by clinical sequelae of fluid loss into the bowel lumen and third space as well as and septic complications in severe cases, which supports increased bowel wall permeability not only for plasma but also for bacteria. Impaired intestinal barrier function has been verified by electrophysiological measurements in surgical intestinal specimens from patients with ulcerative colitis in vitro [22, 35, 36]. The barrier defect occurs especially in the paracellular region, i.e., affecting primarily the sealing function of tight junctions. Analogous changes were detected with the same electrophysiological methods used in TNBS colitis [21]. Improvement in the electrophysiological parameter correlated with impaired bacterial translocation in another model [37], while the latest clinical measurements (in bowel specimens or biopsies) agree with clinical colitis scores (unpublished data).

Our measurements actually showed that improved microcirculation in the early phase of TNBS colitis is associated with stabilized barrier function. The conclusion can be drawn (particularly with the detection of comparable histomorphological changes in both experimental groups) that improved intestinal microcirculation ameliorates the functional sequelae of colitis. In the meantime, similar results or conclusions have also been published by other study groups. In a TNBS colitis model, Friess et al. [38] determined a reduction in mucosal myeloperoxidase activity after improving microcirculation by heparin therapy as a sign of attenuated colitis.

In summary, we found (a) significant impairment of colonic capillary blood early in TNBS colitis in the rat and (b) enhanced gut barrier function associated with improved colonic capillary blood flow in the same model. These findings suggest that (a) microcirculatory disturbances precede histological changes, and (b) that the role of intestinal microcirculation in the evolution of IBD is still underestimated. Although these results may not conclusively answer our questions on the role of microcirculation in IBD, it seems worthwhile to procede with research in this field because an important piece in the puzzle of the pathogenesis of IBD and potentially therapeutic vasoactive substances may otherwise be overlooked.

References

1. Fiocchi C (1998) Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology 115:182–205
2. Carr ND, Pullan BR, Schofield PF (1986) Microvascular studies in non-specific inflammatory bowel disease. Gut 27:542–549
3. Wakefield AJ, Sawyerr AM, Dhillon AP, Pittilo RM, Rowles PM, Lewis AAM, Pounder RE (1989) Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. Lancet 334: 1057–1062
4. Reynolds DG (1971) Injections techniques in the study of intestinal vasculature under normal conditions and in ulcerative colitis. In: Boley SC, Schwartz SS, Williams LF (eds) Vascular disorders of the intestine. Butterworths, London, pp 383–395
5. Brahme F, Lindström C (1970) A comparative radiographic and pathological study of intestinal vasoarchitecture in Crohn's disease and in ulcerative colitis. Gut 11:928–940
6. Lunderquist AL, Lunderquist A (1967) Angiography in ulcerative colitis. Am J Roentgenol 99:18–23
7. Lunderquist AL, Lunderquist A, Knutson H (1967) Angiography in Crohn's disease of the small bowel and colon. Am J Roentgenol 101:338–344
8. Johansson H, Krause U, Olding L (1972) Microangiographic studies in Crohn's disease and ulcerative colitis. Acta Chir Scand 138:409–414
9. Kruschewski M, Dörner A, Busch C, Lierse W (1999) Scanning electron microscopic and light microscopic examinations of the colonic angioarchitecture in Crohn's disease and ulcerative colitis. Int J Colorectal Dis (in press)
10. Foitzik T, Hotz HG, Kinzig M, Sorgel F, Buhr HJ (1997) Influence of changes in pancreatic tissue morphology and capillary blood flow on antibiotic tissue concentrations in the pancreas during the progression of acute pancreatitis. Gut 40:526–530
11. Hotz HG, Foitzik T, Rohweder J, Schulzke JD, Fromm M, Runkel NSF, Buhr HJ (1998) Intestinal microcirculation and gut permeability in acute pancreatitis. Early changes and therapeutic implications. J Gastrointest Surgery 2:518–525

12. Schneider P, Foitzik T, Kahrau S, Buhr HJ (1999) Etablierung der Intravitalmikroskopie der Lunge am Rattenmodell. Langenbecks Arch Chir [Suppl] (in press)
13. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL (1989) Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96: 795–803
14. Klyscz T, Jünger M, Jung F, Zeintl H (1997) Cap Image – ein neuartiges computergestütztes Videobildanalysesystem für die dynamische Kapillarmikroskopie. Biomed Technik 42: 168–175
15. Mithöfer K, Schmidt J, Gebhard MM, Buhr HJ, Herfarth Ch, Klar E (1995) Measurement of blood flow in pancreatic exchange capillaries with FITC- labeled erythrocytes. Microvasc 49: 33–48
16. Fromm M, Schulzke JD, Hegel U (1985) Epithelial and subepithelial contributions to transmural electrical resistance of intact rat jejunum in vitro. Plugers Arch 405: 400–402
17. Schulzke JD, Fromm M, Hegel U (1986) Epithelial and subepithelial resistance of rat large intestine: segmental differences, effect of stripping, time course, and action of aldosterone. Plugers Arch 407: 632–637
18. Kruschewski M, Rohweder J, Foitzik T, Hotz H, Buhr HJ (1997) Nachweis signifikanter Störungen der Darmmikrozirkulation bei verschiedenen Colitis-Modellen. Langenbecks Arch Chir [Suppl 1]: 435–438
19. Foitzik T, Hotz HG, Kinzig M, Sorgel F, Klar E, Warshaw AL, Buhr HJ (1996) Improvement of pancreatic capillary blood flow does not augment the pancreatic tissue concentration of imipenem in acute experimental pancreatitis. Eur Surg Res 28: 395–401
20. Messmer K, Kreimeier U (1989) Microcirculatory therapy in shock. Resuscitation 18 Suppl: S51–61
21. Rohweder J, Foitzik T, Runkel N, Fromm M, Schulzke JD, Buhr HJ (1997) In vitro-Charakterisierung der intestinalen Barrierefunktion an zwei experimentellen Colitis-Modellen der Ratte. Langenbecks Arch Chir [Suppl 1]: 4439–4443
22. Schmitz H, Barmeyer C, Fromm M, Riecken EO, Schulzke JD (1996) Diarrheal mechanism in ulcerative colitis: epithelial barrier defect and impaired ion transport. Gastroenterology 110: A358
23. Guslandi M, Polli D, Sorghi M, Tittobello A (1995) Rectal blood flow in ulcerative colitis. Am J Gastroenterol 90: 579–580
24. Leung FW, Su KC, Yonei Y, Passaro E, Guth PH (1993) Regional differences in mucosal hemodynamics in experimental colonic injury in rats. Dig Dis Sci 38: 1220–1223
25. Panés J, Granger DN (1998) Leukocyte-endothelial cell interactions: molecular mechanism´and implications in gastrointestinal disease. Gastroenterology 114: 1066–1090
26. Wolff KD, Marks C, Uekermann B, Specht M, Frank KH (1996) Monitoring of flaps by measurement of intracapillary haemoglobin oxygenation with EMPHO II: experimental and clinical study. Br J Oral Maxillofac Surg 34: 524–529
27. Kawano S, Tsuji S, Hayaski N, Takei Y, Nagano K, Fusamoto H, Kamada T (1995) Effect of loxoprofen sodium, a newly synthesized non-steroidal anti-inflammatory drug, and indomethacin on gastric mucosal haemodynamics. J Gastroenterol Hepatol 16: 81–85
28. Elizalde JI, Hernandez C, Llach J, Monton C, Bordas JM, Pique JM, Torres A (1998) Gastric intramucosal acidosis in mechanically ventilated patients: role of mucosal blood flow. Crit Care Med 26: 827–832
29. Su KC, Leung FW, Guth PH (1989) Assessment of mucosal hemodynamics in normal human colon and patients with inflammatory bowel disease. Gastrointest Endosc 35: 22–27
30. Tsujii M, Kawano S, Tsuji S, Kobayashi I, Takei Y, Nagano K, Fusamoto H, Kamada T, Ogihara T, Sato N (1995) Colonic mucosal hemodynamics and tissue oxygenation in patients with ulcerative colitis: Investigation by organ reflectance spectrophotometry. J Gastroenterol 30: 183–188
31. Elson CO, Sartor RB, Tennyson GS, Riddell RH (1995) Experimental models of inflammatory bowel disease. Gastroenterology 109: 1344–1367
32. Leung FW, Koo A (1991) Mucosal vascular stasis precedes loss of viability of endothelial cells in rat acetic acid colitis. Dig Dis Sci 36: 727–732
33. Rutgeerts P, Geboes K, Vanztrappen G, Beyls J, Sachar DB, Wolfson DM, Greenstein AJ (1983) Risk factors for postoperative recurrence of Crohn's disease. Gastroenterology 85: 917–921
34. Buckell NA, Williams GT, Bartram CI, Lennard-Jones JE (1980) Depth of ulceration in acute colitis: correlation with outcome and clinical and radiologic features. Gastroenterology 79: 19–25
35. Volk EE, Shapiro BD, Easley KA, Goldblum JR (1998) The clinical significance of a biopsy-based diagnosis of focal active colitis: a clinicopathologic study of 31 cases. Mod Pathol 11: 789–794
36. Gardiner KR, Anderson NH, Rowlands BJ, Barbul A (1995) Colitis and mucosal barrier dysfunction. Gut 37: 530–535
37. Foitzik T, Stufler M, Hotz HG, Klinnert J, Wagner J, Warshaw Al, Schulzke JD, Fromm M, Buhr HJ (1997) Glutamine stabilizes intestinal permeability and reduces pancreatic infection in acute experimental pancreatitis. J Gastrointest Surg 1: 40–47
38. Friess W, Pagiaro E, Canova E, Carraro P, Gasparini G, Pomerri F, Martin A, Carlatto C, Mazzon E, Sturniolo GC, Longo G (1998) The effect of heparin on trinitrobenzene sulphonic acid-induced colitis in the rat. Aliment Pharmacol Ther 12: 229–236