Changes of Colonic Mucosal Microcirculation and Histology in Two Colitis Models
An Experimental Study Using Intravital Microscopy and a New Histological Scoring System

MARTIN KRUSCHEWSKI, MD,* THOMAS FOITZIK, PhD,* ALEXANDRA PEREZ-CANTÓ, MD,† ANNETTE HÜBOTTER, MD,† and HEINZ JOHANNES BUHR, PhD*

This study investigated capillary blood flow (CBF) and pathomorphological alterations in the mucosa of different bowel segments at different times after disease onset in rats with colitis induced by either trinitrobenzensulfonic acid (TNBS) or mitomycin-C. CBF was determined by intravital microscopy using fluorescein-labeled erythrocytes. The histological degree of inflammation was assessed by a new scoring system. Severe acute histological changes were found in the distal colon 24 hr after induction of TNBS colitis (score: 8.9 ± 1.0). CBF was increased (2.9 ± 0.05 vs. 2.6 ± 0.04 nl/min in healthy controls). The histological alterations persisted until day 3 (8.5 ± 0.9) when CBF significantly decreased (1.8 ± 0.05 nl/min). After 15 days, moderate acute inflammation was still detectable histologically (5.4 ± 1.3), but CBF had returned to normal values. In mitomycin-C colitis, changes developed mainly in the proximal colon: After three days, there was mild inflammation (2.8 ± 1.2) with normal CBF (2.5 ± 0.1 nl/min). After seven days, the inflammation had increased (4.8 ± 1.1), while CBF had decreased (1.5 ± 0.06 nl/min). These changes persisted for six weeks (5.3 ± 0.7; 1.2 ± 0.05 nl/min). These data suggest that disturbed colonic microcirculation may play an important role in the pathogenesis of inflammatory bowel disease regardless of the histopathomorphological alterations.

KEY WORDS: microcirculation; intravital microscopy; histological scoring system; TNBS colitis; mitomycin colitis; inflammatory bowel disease.

Altered intestinal wall vascularization and disturbed microcirculation have been repeatedly discussed in various studies on the etiology and pathogenesis of chronic inflammatory bowel disease (IBD) (1, 2). However, their role in the multifactorial pathogenetic course is unclear and is usually considered secondary to histological changes. The problem is that the topic has not yet been sufficiently investigated. There are only a few angiographic, microangiographic, and anatomic corrosion studies that describe the morphology, and there are even fewer functional investigations (3–10). These studies describe blood flow either in vitro in surgical samples (11) or in vivo in patients by endoscopic laser Doppler flowmetry (12) or (endoscopic) spectrophotometry (13–15). The gold standard for microcirculatory investigations is intravital microscopy. For methodological reasons, this method is not applicable in the human bowel, so these questions cannot be answered without animal experi-
ments. Apart from a study by Leung and Koo (16), who observed uniform stasis of the red blood cells in the mucosal capillaries minutes after contact of the colonic mucosa with 10% acetic acid, direct intravital microscopic studies have not yet been done. Therefore, the aim of this study was to systematically investigate colonic mucosal microcirculation by performing intravital microscopy at different times in two established models of IBD. The findings were compared with pathomorphological changes. The degree of inflammation was assessed by a newly developed histological scoring system.

We selected two established IBD models (reviewed in 17): the TNBS colitis model (18) and the mitomycin-C colitis model (19). The TNBS colitis model was selected for comparison with Crohn’s disease because it involves a localized inflammatory reaction in the distal colon as well as regular development of granulomas. In contrast, mitomycin-C colitis was selected because it leads to mucosa-restricted pancolitis, as in ulcerative colitis.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (330g ± 20g) were used in this study. The tests were approved by the Berlin Senate Administration for Health and Social Affairs based on §8 of the Animal Protection Laws (No. 0311/95). The animals were kept in a room under controlled temperature (23°C) and automatic day–night rhythm (12 hr). Each cage housed a maximum of four animals. The animals had free access to food up to 36 hr before colitis induction or intravital microscopy. Access to water was not restricted.

Experimental Colitis. Colitis was induced under ether anesthesia in both models. In the TNBS model, the animals were given a single intrarectal application of 0.25 ml trinitrobenzensulfonic acid in the Trendelenburg position based on the protocol of Morris et al (18). In the second model, the animals received a single intraperitoneal injection of 3.25 mg/kg body wt mitomycin-C based on the description by Keshavarzian et al (19).

Experimental Design. The method was initially assessed and standardized by intravital microscopic evaluation of different intestinal segments in a total of 32 animals (the pilot group). Microcirculation was studied in the terminal ileum as well as proximal and distal colon.

In the TNBS colitis group (group I), intravital microscopy was done in both the proximal and distal colon 24 hr, 3 days, and 15 days after colitis induction in groups of eight animals each. Intravital microscopy in the mitomycin-C colitis group (group II) was also performed in the proximal as well as distal colon 24 hr, 3 days, and 6 weeks after colitis inductions in groups of eight animals each.

On the corresponding days, the proximal and distal colon of eight control animals (control group) were examined (intrarectal or intraperitoneal saline injection).

Preparation Technique. The neck and abdomen were shaved following anesthesia with intraperitoneal pentobarbital Na (17 mg/kg body wt) and i.m. ketamine (142 mg/kg body wt). Polyethylene catheters (0.5 mm ID) were then introduced in the right internal jugular vein and the left common carotid artery for further anesthetization with pentobarbital Na as well as blood pressure measurements and arterial blood gas analysis. Following median laparotomy and atraumatic mobilization in the avascular zone of the proximal and distal colon, the colon segment was exteriorized in front of the abdominal wall and placed on a special stage for intravital microscopic determination of capillary blood flow. Antimesenterial opening of the colon in the almost completely avascular zone was done using microscissors. The colonic wall was supplied with fine sutures and loosely spread for intravital microscopy of mucosal capillary blood flow (CBF) (Figure 1). After examining the first bowel segment, the segment was replaced into the abdominal cavity, and the other segment was mobilized and prepared as described above. The order of examination of the bowel segments (proximal and distal colon) was randomized to avoid systemic errors.

Intravital Microscopy. Arterial blood gas analysis was followed by injection of erythrocytes (0.5 ml/kg body wt)