The use of colonoscopy to follow the inflammatory time course of TNBS colitis in rats

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Abstract

Background and study aims : **Animal models of colitis are widely used to study the pathogenesis of inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS). However techniques allowing sequential assessment of colonic inflammation over time, without the need to sacrifice the animal, are required. This study evaluated *in vivo* colonoscopy to follow the evolution of colitis in rats in comparison with the more commonly used post-mortem macroscopic, microscopic and biochemical assays of inflammation.**

Methods : **Colitis was induced in rats by a single intrarectal instillation of trinitrobenzene sulphonic acid (TNBS). Using a baby upper gastrointestinal endoscope, the severity of colitis was monitored at days 3, 10, 28 and 56 after the induction of colitis. Inflammation was scored by colonoscopy based on the degree of ulceration, extent of inflammation, mucosal bleeding, oedema and stenosis. During follow-up, rats were randomly selected for post-mortem macroscopic and microscopic histology and myeloperoxidase (MPO) assessment of the colon.**

Results : **Colonoscopy showed signs of severe mucosal inflammation in the distal colon 3 days after induction of TNBS colitis. Subsequently, colitis subsided at days 10 and 28 with complete endoscopic remission at day 56. During the acute phase of inflammation, endoscopic findings were consistent with the post-mortem inflammatory parameters (macroscopic and microscopic histopathology, MPO colonic activity). A strong correlation between endoscopy and macroscopy remained even during the chronic phase of inflammation.**

Conclusions : **Our findings suggest that routine endoscopy is a reliable method for monitoring the development and follow-up of the degree of TNBS colitis in rats.** (Acta gastroenterol. belg., **2011**, 74, **304-311**).

Key words : colitis, colonoscopy, inflammation, rat, trinitrobenzene sulphonic acid.

Introduction

Inflammatory bowel diseases (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC), are relapsing chronic inflammatory disorders that may affect the total gastrointestinal tract (CD) or are limited to the colon (UC) (1,2). The pathophysiology of IBD and the effect of new emerging therapies are being examined extensively by numerous animal models of inflammation (3,4). Recent evidence from both human and animal models, suggests that inflammation is not only involved in IBD but also in functional gastrointestinal disorders, such as the irritable bowel syndrome (IBS) (5). Approximately 7% to 31% of IBS patients develop their symptoms after an episode of acute gastroenteritis (6,7,8,9,10). Additionally, data from animal studies demonstrated that colonic hypersensitivity, a key feature of IBS, can persist long after an episode of acute colitis (11,12,13). These data further expand the role of inflammatory animal models in the study of a broad range of inflammatory and functional gastrointestinal diseases.

Unfortunately, evaluating the course of inflammation in rodents – certainly at the long-term – requires serial animal sacrifice, since the inflammatory degree is usually assessed by post-mortem study of macroscopic and microscopic tissue damage and myeloperoxidase (MPO) activity. The indirect inflammatory parameters that can be followed *in vivo* include changes in body weight, physical appearance and behaviour (13). However, this approach lacks validation because no direct indications of inflammation are monitored. In contrast, in clinical practice endoscopy is one of the keystone diagnostic techniques allowing follow-up and management of gastrointestinal inflammation (14). The aim of the present study was to assess the efficacy of colonoscopy as a monitoring tool of inflammation in an experimental colitis model in the rat. To confirm the accuracy and practical use of colonoscopy for the evaluation of colitis, we compared endoscopic findings with post-mortem macroscopic and microscopic histopathological findings and a quantitative myeloperoxidase (MPO) assay in the commonly used rat model of TNBS colitis. We used this model, since the TNBS inflammatory response in the colon is well documented, highly reproducible and has already validated macroscopic and histological scoring systems (15,3,16).

Materials and methods

Animal model & experimental design

Distal TNBS colitis was induced in female Wistar rats (200-240 g) according to published methods (16,15,17). Briefly, rats were fasted for 24 h with free access to drinking water and randomized into control (n = 16) or TNBS (n = 20) groups. After pentobarbital anaesthesia

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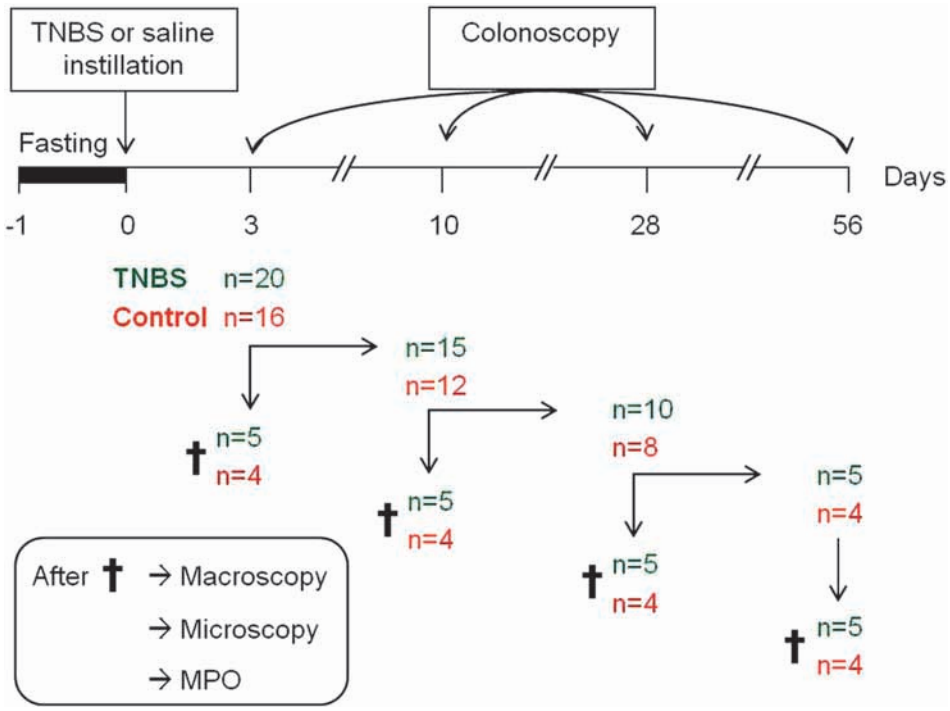

Fig. 1. — Experimental set-up of the follow-up study. Rats were instilled intrarectally with a single dose of TNBS (7.5 mg TNBS dissolved in 40% ethanol, n = 20, blue) or saline (n = 16, red) after a 24 h fasting period. Next, rats were examined by colonoscopy at various time points (3, 10, 28, 56 days). During follow-up, a subgroup of rats instilled with either TNBS (n = 5) or saline (n = 4) were killed (†) after colonoscopy at each evaluated time point for further tissue analysis (macroscopy, microscopy, MPO).

(45 mg kg^{-1} I.P.), a single dose of 0.5 ml of isotonic saline was administered in the colorectum of the control animals through a flexible catheter (18 Gauche) of 4.5 cm length. A single dose of 0.5 ml mixture of 7.5 mg trinitrobenzene sulphonic acid (TNBS) dissolved in 40% ethanol was administered in the colorectum of the TNBS animals. Ethanol breaks the mucosal barrier and is a crucial component of the model.

Rats were kept in a tail-up position for 1 min to prevent immediate expulsion of saline or TNBS solution and then allowed to recover in their cages with free access to food and water. A follow-up study was designed as indicated in Figure 1. At days 3, 10, 28 and 56 after the induction of colitis, colonoscopy was performed under pentobarbital anaesthesia (45 mg kg^{-1} I.P.) and rats were allowed to recover. At each time point, rats of each group were randomly selected for post-mortem examination of the colon and sacrificed by an overdose of pentobarbital (100 mg kg^{-1} I.P.). The distal part of the colon was removed and carefully opened along the mesenteric border to look at gross mucosal damage. Subsequently, this part of the colon was divided in 2 segments along the longitudinal axis : one segment for microscopic histological analysis and one segment for MPO assessment.

Colonoscopic examination

Colonoscopy was performed with a baby upper gastrointestinal Olympus GIF-N30 fiberscope with outer diameter 5.2 mm and a 2.0 mm working channel (Olympus Europa GmbH, Hamburg, Germany). After a 24 h fasting period with free access to drinking water, rats were anaesthetised with a bolus of pentobarbital (45 mg kg^{-1} I.P.) and placed in a supine position. A drop of lubricating jelly (RMS-Endoscopy, St. Martens-Lennik, Belgium) was applied on the anal sphincter to facilitate insertion of the scope. The endoscope was then gently passed through the anus and under endoscopic vision further introduced (Fig. 2A). Remaining faeces were flushed away by injecting water through the endoscope's working channel. Occasionally the colon was inflated with air for better visualisation of the lumen. The tip of the endoscope could be introduced up to 10 cm proximal from the anus (Fig. 2B). During withdrawal of the endoscope, the mucosal damage was scored endoscopically using a blinded code concerning the treatment group. An endoscopic grading scale to determine mucosal inflammation was adapted from existing clinical and animal experimental scoring systems (18,19,20,21,22,23). The degree of inflammation, extent of disease, oedema, stenosis and bleeding were scored separately and a final score was given by summation of these subscores (Table 1). Pictures were captured with a digital camera connected to the endoscope. On average, it took about 3 min per rat to insert the endoscope and perform colonoscopic scoring. Rats were placed under surveillance during recovery and were returned to their cages when regaining consciousness.

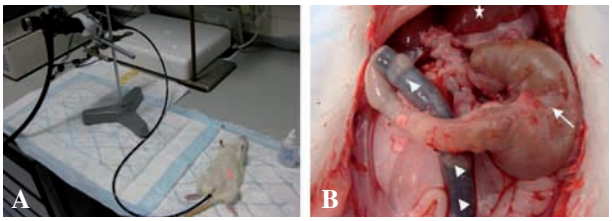

Fig. 2. — Use of the baby endoscope in female Wistar rat (200-220 g). Figure A shows the inserted scope, the red dot indicating the tip of the scope. Figure B illustrates anatomical location of the endoscope (tip is at 10 cm proximal to the anus) in the colon (white arrowheads) after laparotomy and further removal of the small intestine for optimal visualisation. Caecum (white arrow) and liver (white asterix) are also shown.

Macroscopic examination

Rats were randomly sacrificed at different time points (Fig. 1). After sacrifice, the colon was collected and rinsed with ice-cold Krebs solution. The colon was opened longitudinally and pinned out on a Petri dish to examine colonic mucosal damage. The mucosal surface of the distal colon was inspected with a binocular (Zeiss Jena, Jena, Germany) and the extent of the inflammation was verified using a standardized scoring system ranging from 0 (no inflammation) to 10 (severe damage extending over > 5 cm) (Table 2) (15).

Microscopic examination

Full-thickness samples of approximately 1 cm were taken from areas of the distal colon at areas where signs of inflammation were observed during macroscopy. Segments were fixed in 4% formaldehyde for 24 h, embedded in paraffin and cross sections of 5 µm were stained with haematoxylin and eosin (H&E). To grade the inflammation, we used a commonly used histological damage score that includes the following parameters : inflammatory infiltrate, number of gut wall layers infiltrated, loss of mucosal architecture and oedema (24,25). The total score ranged from a minimum of 0 to a maximum of 10 (Table 3). Histological sections were examined using a conventional microscope (Olympus BX40, Melville, New York, USA).

Myeloperoxidase tissue activity

MPO activity was assayed according to a previously published method used in our group (26,27). In brief, full-thickness tissue samples were harvested from the distal colon. Samples were taken from areas where signs of inflammation were observed during macroscopy. Samples were blotted dry and placed in a potassium phosphate buffer pH 6.0 containing 0.5% hexadecyltrimethylammonium bromide (5 g tissue per 100 mL buffer). The samples were placed on ice, homogenized for 30 s and subjected to two sonication and freeze-thawing cycles. The suspension was centrifuged at 15000 g for 15 min at 4°C. Aliquots (0.1 mL) of the supernatant were added to 2.9 mL of *o*-dianisidine solution (16.7 mg of *o*-dianisidine in 1 mL methanol, 98 mL 50 $mmol^{-1}$ potassium phosphate buffer pH 6.0 and 1 mL of a 0.05 % H_2O_2 solution as a substrate for MPO enzyme). The change in absorbance was read at 460 nm over 60 s using a Spectronic Genesys 5 spectrophotometer (Milton Roy, NY, USA). One unit of MPO activity was defined as the quantity able to convert 1 µmol H_2O_2 to H_2O per min at 25°C and was expressed as units per gram tissue (U gram $tissue^{-1}$).

Table 1. — **Colonoscopic scoring system**

Colonoscopic finding	**Score**
Degree of inflammation	**0-6**
Normal aspect of the mucosa	0
Hyperaemia	1
Ulceration occupying the lumen maximally up to ¼ of circumference	2
Ulceration occupying the lumen between ¼ - ½ of circumference	3
Ulceration occupying the lumen > ½ but not all around	4
Circular ulcer	5
Circular ulcer with longitudinal extension	6
Extent of disease (max 10 cm)	**cm**
Stenosis	**0-1**
Absent	0
Present	1
Oedema	**0-1**
Absent	0
Present	1
Active bleeding	**0-1**
Absent	0
Present	1

Colonoscopic scoring criteria for TNBS-induced colitis. Five parameters are taken into account : degree of inflammation (0-6), extent of disease (cm), presence of stenosis (0-1), oedema (0-1) and active bleeding (0-1). The total score is given by summation of these individual scores ranging from 0 to 19, with a higher score indicating higher disease activity.

Table 2. — **Macroscopic scoring system adapted from Wallace and Keenan, 1990**

Macroscopic finding	Score
Normal aspect of the mucosa	0
Localised hyperaemia, no ulcers	1
Ulceration without hyperaemia/bowel wall thickening (inflammation)	2
Ulceration with hyperaemia/bowel wall thickening (inflammation) at 1 site	3
2 or more sites of ulceration and hyperaemia/bowel wall thickening (inflammation)	4
Major sites of damage extending > 1 cm along length of colon	5
When an area of damage extended > 2 cm along length of colon, score was increased by 1 for each additional cm of involvement	6-10

Macroscopic scoring criteria for TNBS-induced colitis. Macroscopically visible damage was scored on a 0-10 scale using the scoring system described in Wallace and Keenan, 1990. Grading of colitis is based on ulceration, inflammation and extent of disease.

Table 3. — **Microscopic scoring system adapted from Hunter *et al.*, 2005**

Histologic finding	Normal	Minimal	Mild	Maximal
Inflammatory infiltrate	0	1	2	3
Number of layers infiltrated	0	1	2	3
Mucosal tissue damage	0	1	2	3
Mucosal oedema	Absent = 0 Present = 1			

Histological scoring criteria for TNBS-induced colitis. Four parameters were taken into account : inflammatory infiltrate (0-3), number of gut wall layers infiltrated (0-3), loss of mucosal architecture (0-3) and oedema (0-1). The total score ranged from a minimum of 0 to a maximum of 10.

Solutions and drugs

TNBS was obtained from Fluka, Neu Ulm, Germany. Pentobarbital (Nembutal®) from Ceva, Brussels, Belgium. Hexadecyltrimethyl-ammonium bromide and *o*-dianisidine dihydrochloride from Sigma-Aldrich Inc., St Louis, MO, USA. Hydrogen peroxide from Merck, Darmstadt, Germany. Formaldehyde and haematoxylin from Merck, Darmstadt, Germany. Eosin from Acros Organics, New Jersey, USA. Paraffin from McCormick, St. Louis, Missouri, USA.

Presentation of results and statistical analysis

All data are presented as mean ± S.E.M. for n the number of rats. For statistical analysis all values were analysed by a two-way ANOVA with Student-Newman-Keuls post hoc analysis. $P < 0.05$ was considered statistically significant. Correlations were analysed using Pearson's correlation test and were considered statistically significant at $P < 0.05$. Data were analysed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.00 (GraphPad Software Inc., San Diego, CA, USA).

Ethical approval of the study

All animal experiments were performed in accordance with the guidelines of the Committee for Medical Ethics and the use of Experimental Animals at the University of Antwerp that approved the study protocol (file number 2008-01).

Results

Follow-up by colonoscopy

Rats that had received a saline instillation (n = 16) showed a smooth and shiny mucosa with normal blood vessel architecture when examined with the endoscope (Fig. 3A). No differences between the evaluated time points (3-56 days) could be detected during follow-up of control animals (Fig. 4A). In contrast, rats treated with TNBS (n = 20) developed overt colorectal mucosal inflammation, characterised by severe ulceration, oedema, stenosis and bleeding (Fig. 3B-J) when examined 3 days after TNBS instillation. The mean endoscopic score was 11 ± 0.5 (n = 20). In 7% of the rats (n = 3/20), stenosis prevented examination of the complete inflamed colonic region. Re-evaluation at 10 days post-TNBS revealed that tissue damage was reduced to a mean endoscopic score of 3.5 ± 0.8 compared to day 3 (Fig. 4A). Although mucosal ulcers and oedema were occasionally seen at this time point, colonoscopic damage scores significantly decreased compared to day 3 mainly because of a decreased disease severity and extent of inflammation In 13% (n = 2/15) of rats, colonoscopy could not detect any signs of inflammation at day 10. At day 28, mean endoscopic score was 0.5 ± 0.3 and remnants of mucosal injury was seen in 30% of rats (n = 3/10) (Fig. 4A). At this time point, inflammation was mild, since only signs of hyperaemia were found and ulcers or other severe inflammatory features were no longer apparent. Colonoscopy indicated that colitis was in

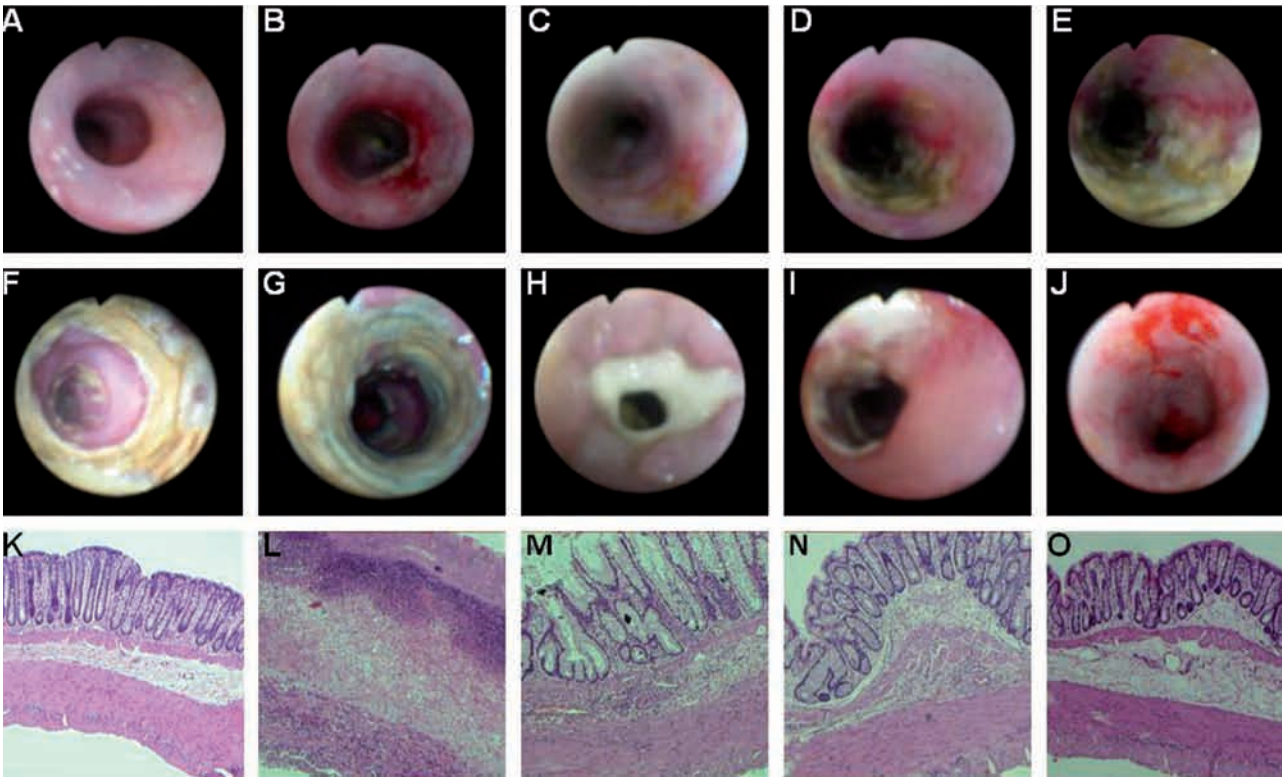

Fig. 3. — Representative pictures of *in vivo* colonoscopy of TNBS colitis in rats (A-J). The following parameters were included in the scoring system : (A) Normal aspect of mucosal surface, (B) hyperaemia, (C) ulceration occupying lumen up to ¼, (D) ulceration occupying lumen more than ¼ but less than ½, (E) ulceration occupying lumen more than ½ but not all around, (F) circular ulcus, (G) circular ulcus with longitudinal extension, (H) stenosis, (I) oedema and (J) bleeding. H&E stains of colon sections (×20) illustrate severe damage at all layers during acute inflammation (L) (3 days) compared to control (K). Long-term assessment showed resolution of colitis characterised by mucosal tissue repair at day 10 (M), with a mild infiltrate in the submucosal layers. At day 28 a mild infiltrate was apparent (N) and there was complete remission at day 56 (O).

complete remission in all remaining animals (n = 5) at 56 days after TNBS induction (Fig. 4A).

As a result of this study, 5/20 rats with TNBS colitis were ultimately evaluated by repeated colonoscopy throughout the entire inflammatory course (day 3 to 56).

Macroscopic tissue assessment

Three days after TNBS administration, macroscopic mucosal inflammation resembled colonoscopic injury in terms of ulceration, oedema and mucosal bleeding reaching a mean score of 7.2 ± 0.9 (Fig. 4B). At day 10, the macroscopic damage followed the findings of colonoscopy : focal ulcers accompanied by hyperaemia and oedema were significantly (Fig. 4B) decreased in comparison with day 3 but still occasionally seen. No prominent mucosal injuries were present 28 days post-colitis and completely absent 56 days post-colitis (Fig. 4B). At all time points examined, macroscopy closely correlated with endoscopic alterations (Pearson correlation day 3 : r= 0.97, day 10 : r = 0.92, day 28 : r = 0.84).

Microscopic tissue assessment

The histological appearance of control tissue did not show damage (Fig. 3K) whereas acutely inflamed (day 3) colonic tissue samples showed severe transmural inflammation, characterized by multifocal areas of necrosis, total destruction or ablation of the mucosal architecture and massive infiltration of polymorphonuclear leukocytes within the colonic layers (Fig. 3L). The mean score at day 3 was 8.6 ± 0.2 (Fig. 4C). At day 10, histology showed healing of the mucosal lesions while an increased cell infiltrate remained (Fig. 3M). Twenty-eight days after colitis, histological sections showed subtle signs of inflammation such as mucosal crypt distortion, muscular layer thickening and a mild increased infiltrate of immune cells (Fig. 3N). No signs of inflammation could be seen at day 56 in all rats examined (Fig. 3O, 4C). Histological assessment of the acute inflammatory response correlated well with the colonoscopy scoring (Pearson correlation day 3 : r = 0.99). However, in the course of the colitis (day 10 to 28) the correlation coefficient decreased (day 10 : r = 0.84 ; day 28 : r = 0.77).

Myeloperoxidase activity assay

Acute inflammation within 3 days after TNBS administration was confirmed by a significantly increased colonic MPO activity compared to control activity levels (Fig. 4D). At 10 days of colitis, MPO activity tended to

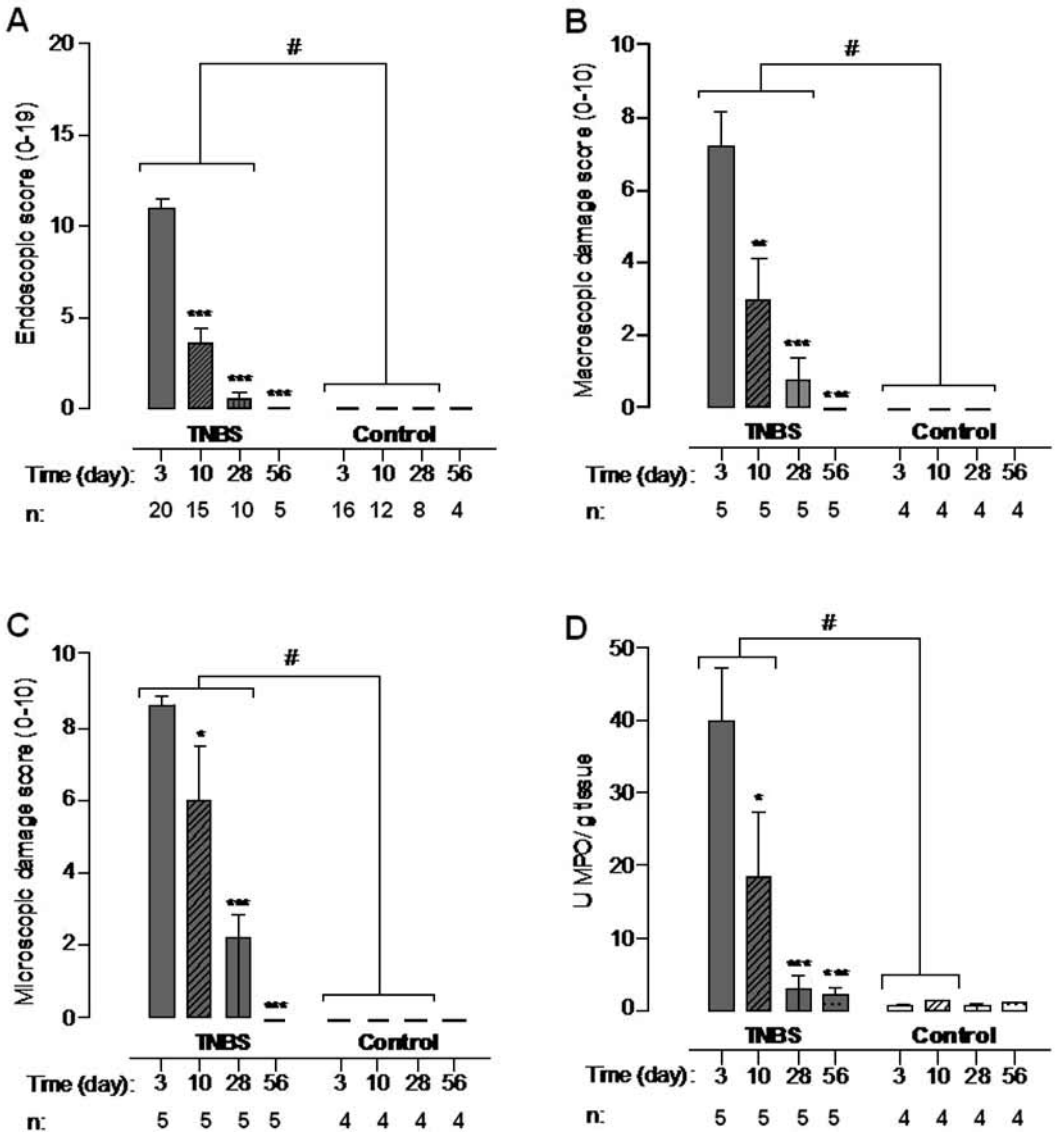

Fig. 4. — Time course of the inflammatory parameters. (A) Temporal changes in colitis severity as assessed by colonoscopy. Animals treated with either TNBS or saline were examined endoscopically at days 3, 10, 28 and 56. (B) Macroscopic scores, (C) microscopic scores and (D) MPO activity for animals sacrificed at days 3, 10, 28 and 56. Data are presented as mean ± S.E.M. Data were analysed by two-way ANOVA with post-hoc Student-Newman-Keuls analysis ; #$P < 0.05$, significantly different from control groups, *$P < 0.05$, significantly different from the day 3 TNBS group.

be lowered but was still significantly higher than in control tissue (Fig. 4D). Twenty-eight and 56 days post-TNBS, MPO activity was not significantly different compared to controls (Fig. 4D). Correlations with the quantitative MPO activity index of inflammation, although significant at day 3 and 10 (Pearson correlation day 3 : r = 0.91, day 10 : r = 0.70) were not statistically significant at later time points of investigation (Pearson correlation day 28 : r = 0.49).

Discussion

In clinical practice endoscopy is a powerful tool to investigate gastrointestinal disorders such as IBD and IBS. Beyond its diagnostic utility, gastrointestinal endoscopy has an important role in staging the extent and severity of disease, mucosal tissue sampling, in the assessment of the efficacy of medical therapy and in the evaluation and treatment of complications like strictures and fistulas (28,29,14,30). Multiple endoscopic scoring systems and indices have been developed, such as the Crohn's Disease Endoscopic Index of Severity (CDEIS), the Simple Endoscopic Score for Crohn's Disease (SES-CD) and the Mayo score to measure endoscopic disease activity in IBD (18,19,20). Not surprisingly, endoscopy is considered the "golden standard" for the evaluation of disease activity and disease extent in patients with IBD.

Animal models of intestinal inflammation are widely used to unravel the underlying pathogenesis of IBD and IBS. One of the major purposes of experimental basic

research is to develop novel treatment strategies and to perform preclinical drug testing in a translational set-up. One of the major drawbacks however, is the high number of animals that need to be sacrificed to assess the course of inflammation over time. Some efforts have been made to evaluate the validity of fecal markers in rodent models (31) as a non-invasive method to diagnose inflammation. Different studies have shown that fecal calprotectin, although of great interest in clinical practice, could not be determined in the TNBS colitis rat model. Transferrin, a fecal marker evaluated in TNBS colitis rats, is promising, although the validity of this marker in other inflammatory models can be questioned. We strongly believe that clinical methods to evaluate inflammation, like endoscopy, are underused in experimental animal research. Literature data are available concerning in vivo monitoring of the development of experimental gastrointestinal carcinogenesis (21,32,33). Endoscopic validation of gastrointestinal inflammation by endoscopy is however scarcely documented (23) and could nevertheless offer important advantages in the follow-up of the course of inflammation with or without experimental treatment strategies.

Therefore, we endoscopically evaluated the inflammatory course of TNBS colitis, a well-defined and reproducible model of colitis for IBD in the present study. Our main goals were to construct a clinically relevant endoscopic score for animal use and to validate it compared to the current golden standard of macro- and microscopy. In order to obtain an accurate image of the inflammatory appearance of the mucosa, we developed an endoscopic scoring system based on the degree of ulceration, the extent of disease and the presence of oedema, stenosis or spontaneous bleeding. We based this score on existing clinical and experimental scoring systems (18,19,20,21, 22,23) used in clinical diagnosis and follow-up of IBD patients (34). Next, we attempted to validate the use of colonoscopy during the complete course of TNBS colitis in rat by comparing endoscopic findings with classically used inflammatory parameters that include macroscopy, microscopy (histology) and MPO.

Acute colitis was observed 3 days after TNBS instillation and progressively subsided with complete remission after approximately 56 days. This self-limiting character of TNBS colitis in rats was previously shown by macroscopic and microscopic methodologies (16,3) and was endoscopically confirmed in this study as evidenced by the gradual decrease of the endoscopic score over the time course of inflammation. During the acute stage of colitis, the endoscopic scores were well consistent with all the post-mortem scored parameters of inflammation (macroscopic and microscopic tissue damage and MPO activity). When considering the entire inflammatory course (day 3 to 56), we found that the endoscopic scores correlated best with the macroscopic score. Most likely this is due to the fact that both scores assess mucosal tissue damage from a luminal viewpoint. During the healing phase of colitis, microscopy revealed that histological signs of inflammation were still present at day 28 in 67% of the rats, although these rats no longer showed endoscopic signs of inflammation. This discrepancy can be explained by the fact that colonoscopic mucosal activity of inflammation does not reflect the transmural damage that is assessed by microscopic histology. Interestingly, colonic MPO activity was normalised at day 28 and this was in line with the normalised endoscopic and macroscopic scores but not with microscopy. These results suggest that the remaining inflammation at this late time point does not fully consist of the presence of a neutrophilic derived cell infiltrate. We thus developed an endoscopic score allowing the follow-up of colonic inflammation in rats over time that correlates very well with the previous macroscopic standard assessment of mucosal inflammation.

Due to the nature of the follow-up study, it was inevitable to repetitively anesthetise rats with pentobarbital. We are aware that this might affect duration or severity of the inflammatory response. However, to our knowledge, interference with colitis by pentobarbital anaesthesia has not been reported so far in either human or animal studies

When the endoscope is inserted, the colonic wall is stretched a few centimeters. Therefore the number of cm reached by the endoscope does not reflect the real length of the distal colon. We could only examine the distal part of the colon. Reaching the caecum is prevented by a sharp flexura at 10cm from the anus (endoscopic centimeters).

With this study we also provide evidence that rodent endoscopy by using an ultrathin endoscope is feasible and that the procedure is safe on a repeated basis, since multiple colonoscopic examinations in control rats did not cause trauma or mortality as verified by macroscopic, histological as well as MPO analysis. We have no indications to believe that routine endoscopy might cause additional mucosal damage when screening inflamed mucosa (TNBS).

Moreover, preliminary experiments with the Olympus biopsy forceps (EndoJaw FB-231K, Olympus Medical Systems Corp., Tokyo, Japan) in our laboratory have shown that rat colon mucosal tissue sampling is feasible through the endoscope's working channel without risk of perforation (data not shown). Since many animal experiments rely on tissue availability, colonoscopy combined with biopsy offers interesting perspectives for assessing efficacy of novel therapeutic approaches.

In conclusion, our data suggest that colonoscopy in rats is a valid *in vivo* tool for assessing inflammation over a prolonged period of time and on a repeated basis without the need to sacrifice the animal. We additionally validated an endoscopic scoring system of colonic inflammation and proved its accuracy compared to traditional methods of inflammatory assessment such as macroscopy, microscopy and MPO. With this study we propose endoscopy as a safe, simple, reproducible and easy-to-use method to evaluate TNBS colitis in rats and

follow-up the course of experimental colitis on a regular base, allowing a clinically relevant follow-up of novel drug therapies.

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