Life Sciences 236 (2019) 116833

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Changes in colonic contractility in response to inflammatory bowel disease: Long-term assessment in a model of TNBS-induced inflammation in rats

Marcos F.F. Calabresi[a], Alexandre Tanimoto[b], André G. Próspero[a], Fabio P.F. Mello[a], Guilherme Soares[a], Luiz C. Di Stasi[b], José R.A. Miranda[a,*]

[a] Laboratory of Biomagnetism, Department of Physics and Biophysics, Bioscience Institute, São Paulo State University, Botucatu, São Paulo, Brazil
[b] Laboratory of Phytomedicines, Pharmacology and Biotechnology, Department of Pharmacology, Bioscience Institute, São Paulo State University, Botucatu, São Paulo, Brazil

ARTICLE INFO

Keywords:
Inflammatory bowel disease
Colonic contractility
Alternating current biosusceptometry

ABSTRACT

Aims: Inflammatory bowel disease is a chronic relapsing inflammation that affects the gastrointestinal tract, causing changes in colonic motility. The evolution of these changes is not completely understood and possibly related to symptoms that appear in different degrees of the intestinal inflammation. Therefore, our aim is evaluate during 14 days of assessment aspects of colonic contractility using 2,4,6-trinitrobenzenesulfonic acid (TNBS) model of inflammation in rats and associate the inflammatory process with colonic motility.

Methods: Contractility and inflammatory parameters were assessed in the same animal in six different moments: before intestinal inflammation induction, 2, 5, 8, 11, and 14 days after induction. The mechanical activity was determined by alternating current biosusceptometry (ACB) and subdivided into rhythmic propagating ripples (RPR) and rhythmic propulsive motor complexes (RPMC). We assessed inflammation by determining myeloperoxidase activity in feces.

Results: Transient and permanent changes were observed in colonic motility as a function of the inflammatory process evaluated through myeloperoxidase activity. We identified two contraction profiles: RPR and RPMC. The microscopic analysis demonstrated a depth of damage caused by an injury that was associated with changes in motility.

Conclusions: We implemented a robust and adequate (specific) signal processing to quantify two measured colonic frequency patterns. Thus, we performed a detailed temporal analysis of the consequences of TNBS-induced inflammation on colonic motility in rats. Our approach enables further long-term assessments in the same animal with different mechanisms and duration of injury, remission, treatments and their motor consequences.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing, and severe inflammatory process of the gastrointestinal tract (GIT) categorized as ulcerative colitis (UC) and Crohn's disease (CD) [1,2]. The pathogenesis of IBD is not fully understood, and no definitive pharmacological cure is available [1,2]. Pain, diarrhea, bloody stool, and the loss of peristaltic function are standard clinical features and complications of IBD [3]. Gastrointestinal motor disorders become a confounding factor, especially when the inflammation has decreased or is in remission [4].

Several animal models were employed to investigate the pathogenic profile of IBD, where the 2,4,6-trinitrobenzenesulfonic acid (TNBS) is the most used [5,6]. Acting as hapten, the TNBS produces acute colonic necrosis, usually transmural, sharing properties that resembling CD, particularly cytokine secretion patterns, mechanisms of oral tolerance and fibrosis process [7]. In this model, mucosal injury and inflammatory cell infiltration are found shortly after exposure to TNBS, and chronic inflammation and lymphocytic cell infiltration usually occurs 48 h after exposure and persists for a few weeks [8]. Changes in the neurophysiological properties of myenteric neurons that are caused by the disease persist, even after the resolution of inflammation, thus contributing to dysmotility [7,9].

Studies have reported the influence of IBD on intestinal motility [4,10,11] and demonstrated relationships between intestinal

* Corresponding author at: Departamento de Física e Biofísica, UNESP - Campus de Botucatu, Instituto de Biociências, Rua Prof. Dr. Antonio Celso Wagner Zanin, s/n°, CEP: 18618-689 Botucatu, SP, Brazil.
E-mail addresses: mfcalabresi@ibb.unesp.br (M.F.F. Calabresi), alexandre.tanimoto@unesp.br (A. Tanimoto), agprospero@ibb.unesp.br (A.G. Próspero), ldistasi@ibb.unesp.br (L.C. Di Stasi), jmiranda@ibb.unesp.br (J.R.A. Miranda).

https://doi.org/10.1016/j.lfs.2019.116833
Received 14 May 2019; Received in revised form 22 August 2019; Accepted 2 September 2019
Available online 03 September 2019
0024-3205/

inflammation and impairments in local motor function [4,12]. Motility disorders of the GIT have critical clinical importance [2], since they can lead to dysbiosis, induce translocation of bacteria or bacterial products through a dysfunctional mucosal barrier [2], and increase the severity of intestinal injury [8].

Majority of colonic motility studies has been performed using *ex vivo* experiments. Huizinga [13] developed an *ex vivo* study of colon motor activity using pressure transducers and a spatiotemporal map image analysis, classifying the motor activity patterns in rhythmic propulsive motor complexes (RPMCs) and rhythmic propagating ripples (RPRs). RPR are permanent oscillations, with a high frequency and low amplitude, characterized by spontaneous motor patterns, where their functions are related to mixing food and absorption. On the other hand, the RPMC is characterized by strong contractions with low frequency and high amplitude, and is associated with stool propulsion through bursts.

The correlation between inflammation and smooth muscle function has been reported in *ex vivo* studies [8,14]. However, most of these studies did not provide *in vivo* information regarding the complex relationship between inflammatory responses and motility disorders.

Alternating current biosusceptometry (ACB) is a non-invasive technique, extensively employed in gastroenterology assessments in both animal and human studies [15,16]. This system provides a real-time evaluation of the mechanical activity of contractile organs of GIT (*e.g.*, stomach, small intestine, cecum and colon).

Once that few studies evaluate *in vivo* information on colonic motility, the present study was performed to characterize contractile changes in colonic motility in rats with intestinal inflammation induced by trinitrobenzenesulfonic acid (TNBS), a well-stablished model of intestinal inflammation that resembles intestinal inflammation in human. The changes in colonic motility were associated with different degree of intestinal inflammation evidenced by myeloperoxidase (MPO) activity in rat stools. The parameters were monitored up to 14 days after TNBS intestinal inflammation induction, since at this time the colon of the rats ware recovered. This work sought to identify whether different degrees of TNBS-induced intestinal inflammation influence colonic motor activity.

2. Materials and methods

2.1. Animals and ethical considerations

Male Wistar rats (180–250 g; ANILAB, Paulínia, SP, Brazil) were maintained under standard environmental conditions at 21 °C ± 1 °C with a 12 h/12 h light/dark cycle and constant air filtration. All the experimental protocols were approved by the Institute of Biosciences Ethics Committee for Animal Research (CEEA; protocol no. 042/04-CEEA and 502-CEUA). Therefore, the animals were divided in two groups: Group 1 (n = 10) and Group 2 (n = 60).

Group 1 was used for the motility studies and evaluation of myeloperoxidase (MPO) activity in fecal samples. The same 10 animals were kept alive throughout the experiment and were evaluated 24 h prior to TNBS induction and 2, 5, 8, 11 and 14 days thereafter.

Group 2 (60 rats) was used to evaluate the MPO activity and colon tissue histopathological analyses. Due to the fact that this approach required the animal death, group 2 was subdivided into 5 subgroups of 10 animals each, corresponding to the same moments evaluated in Group 1 (24 h prior to induction of TNBS and 2, 5, 8, 11 and 14 days after) (Fig. 1).

The general condition of the animals was monitored daily, however, no analgesic or anti-inflammatory drugs were administrated to rats to avoid influences on the results.

2.2. Experimental planning and induction of colonic inflammation

The experiments were conducted following the timeline showed in Fig. 1A. Five days before the start of the measurements, 10 animals (Group 1) underwent isoflurane anesthesia (4% for induction and 1.5% for maintenance), followed by a medial laparotomy surgery to implant the magnetic marker (ferrite - $MnFe_2O_4$ - nucleus of 0.15 g, φ = 4.2 mm) in the serosa of the colon at the lesion region. (Fig. 1C).

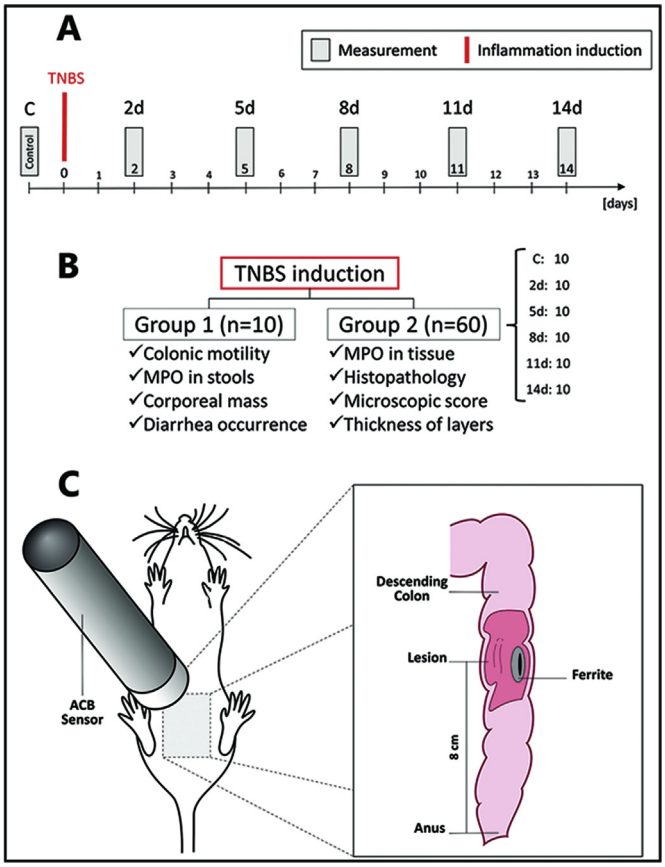

Fig. 1. Experimental design: (A) experimental schedule, indicating motility assessment, inflammation induction, and histopathological analyses; (B) description of groups and assessments; and (C) experimental setup for animal preparation, illustrating the lesion site, ferrite implantation in the lesion, with the sensor positioned on the abdominal surface of the animal.

Twenty-four hours before induction, the first measurements were made for the control animals in group 1. In this group the animals were kept alive throughout the experiment, the control was internal (self-control). For group 2, the animals were killed (decapitation) to obtain the tissues.

In day 0, after the remaining rats were fasted for 12 h, the process of induction of TNBS was performed. According originally described by Morris et al. [6], TNBS (40 mg/ml, 0.25 ml) was administered through a Teflon cannula inserted in the anus of anesthetized (isoflurane) rats kept upside down for 10 min.

Thereafter, the experiment followed the schedule, with the animals in group 1 being measured on days 2, 5, 8, 11 and 14 after induction and respective subgroups of group 2 having their tissues analyzed.

2.3. Assessment of the inflammatory process

To assess the degree of inflammation, we used myeloperoxidase (MPO) activity as a marker of activated neutrophils, according to the method described by Krawisz et al. [17] In the Group 1, MPO activity was assessed in stools [18] collected immediately before each motility assessment, which allowed the animals to remain alive throughout the experiment, and to determine MPO activity as an inflammatory marker in rats feces. For this, the animals were housed individually and the

feces produced up to 2 h before the beginning of the measurement were collected. Rats also had their body mass measured, and occurrence of diarrhea (as detected by perianal fur soiling) was monitored daily. In group 2, the animals were killed at the same time points of the colon samples collection to determine the MPO activity and histopathological analysis.

For MPO activity evaluation samples were suspended in 1 ml of 50 mM phosphate buffer incorporating 0.5% hexadecyltrimethylammonium bromide (pH 6.0), and minced with scissors for 15 s on an ice-cold plate. The resultant suspension was subsequently diluted to a final 1:20 w/v ratio and homogenized for 1 min with an automatic Heidolph homogenizer, sonicated for 10s and subjected to three freeze-thaw cycles. The homogenates were then centrifuged at 7000*g* and 4 °C for 10 min, and the supernatants were assayed for MPO activity. The results are expressed as MPO units per gram of feces for group 1 and MPO units per gram of wet tissue for 2 experiments.

2.4. Colonic motility assessment

Thirty minutes after ingesting 2 g of chow, the animals were anesthetized with isoflurane in an anesthesia system containing an induction box and an inhalation mask, in which the animals were maintained until the end of the motility measurement. The activity signal acquired by ACB was digitized using a multichannel recorder (MP150 System, BIOPAC, Santa Barbara, CA, USA), at a sampling rate of 20 Hz per channel, and stored for further analysis.

The ACB technique employs materials with high magnetic susceptibility as tracers or markers, such as iron manganese oxide (ferrite, $MnFe_2O_4$, 50–100 μm), which provide a high magnetic response and do not require prior magnetization. Ferrite has advantages as a magnetic tracer/marker because it is non-toxic, naturally insoluble and considered inert in the body [16,19].

During the contraction process, the organ moves the marker away from the sensor, which is positioned near the external organ wall, thus decreasing the magnetic signal. Similarly, the relaxation process brings the magnetic marker near the ACB sensor, which increases the signal [15,20]. Thus, ACB allows the recording of mechanical movements of organs of interest, such as a specific region of the colon. ACB has been robustly validated to measure contraction activity compared to gold standard methodologies, such as strain gauge and manometry, presenting a high correlation with these techniques [21] [22].

All of the signals were analyzed and processed using MatLab (Mathworks, Natick, MA, USA), in which were applied moving average (cutoff time between 10 and 60 s to remove low frequencies) and Savitzky Golay (3 order, to smoothers the signals, removing high frequency and/or fast artifacts and maintaining the main characteristics of the signals) filters [20,23]. The main parameter assessed was the colonic contraction frequency, quantified by Fast Fourier Transform (FFT) [11]. Analyzing the FTT spectrum, we observed two prominent peaks which represent predominant contraction frequencies at all moments. The first peak, with a lower frequency (10–30 mHz), is associated with RPMC, and the second peak, with a higher frequency, associated with RPRs (40–110 mHz). In this way, we were able to analyze the frequency variation of each contraction activity throughout the experiment.

2.5. Histopathological analysis

Colon biopsies were collected at 0.5 cm from the colonic damage area and closer to the rectum in all animals of Group 2 (Fig. 1). All slices were fixed in ethanol, formaldehyde and acetic acid (1:9:0.5 v/v). Cross-sections were selected and embedded in paraffin, which were sliced in sections of 5 μm stained with hematoxylin and eosin. In the photomicrographs, we quantified the thickness of tissue layers (mucosa, muscularis mucosae, submucosa, and muscularis externa) and ulcer area, if present. We scored each slice based on the presence of edema, leukocyte infiltration, and connective tissue formation as described by Stucchi et al. [24] and Arribas et al. [25] (see Supplementary File). The images were acquired and analyzed by AxioCam MRc 5 and AxioVision LE software.

2.6. Statistical analysis

The results are expressed as mean ± SEM or as median (range). The statistical analysis of mechanical and MPO activity in stools, colon layer thickness and MPO activity in tissue, were performed using one-way repeated-measures analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test. For the analysis of the ulcer area, we used Student's *t*-test for non-paired samples. To body mass variation, we used a two-way ANOVA followed by Bonferroni multiple-comparison test. To microscopic score, we used the Kruskal-Wallis test followed by Dunn's multiple-comparison test. To diarrhea occurrence, we used the Fischer exact test. Always that possible, the data are presented as differences from control (before inflammation induction). The statistical analyses were performed using GraphPad InStat® version 3.02. Differences were considered significant when $p < 0.05$.

3. Results

Fig. 2 shows MPO activity per gram of stools (Fig. 2A) and per gram of colon tissue (Fig. 2B), body mass variation (Fig. 2C) and a semi-quantitative microscopic score of the histological sections (Fig. 2D). It is possible to note that higher levels of MPO both in stools and tissue were observed 48 h after TNBS induction of intestinal inflammation. After 5 days either in stools or tissue colon, there is a time-dependent decrease in MPO activity. This effect was accompanied, after day 5, by a time-dependent increment in body mass variation and reduced damage microscopic score. These data were also corroborated by the higher percentage of diarrhea in animals, as evidenced 2 days after TNBS induction, with reduced incidence thereafter (Table 1), indicating different degrees of the intestinal inflammatory process induced by TNBS.

Histological micrographs of the colon tissue samples in 6 different moments of the intestinal inflammatory process are presented in Figs. 3 and 4. The normal colon tissue architecture can be observed (Fig. 3A), while several changes in colon epithelium were observed at 2, 5, 8, 11 and 14 days after TNBS induction. Two days after induction of the inflammatory process, ulceration and a huge inflammatory cell infiltration, with mono and polymorphonuclear cells, edema in the submucosa, loss of the mucosa architecture with rupture of the mucosal layer and thickening of both muscularis mucosae and submucosa layers, was found (Fig. 3B). The ulceration spots diminished at day 5, where the inflammatory cell infiltration decreased and the tubular glands in mucosa layer recovered their architecture, but the edema was still as those evidenced at day 2 (Fig. 3C). The edema in submucosa decreased significantly after eight days from TNBS inflammatory induction (Fig. 3D). In the same day, epidermal cells in mucosa were almost fully recovered, and polymorphonuclear cells in submucosal layer were strongly reduced when compared with cell infiltrate at days 2 and 5 (Fig. 3B and C), showing a recovery process in both mucosa and submucosa layers. However, some reminiscent ulceration spots, polymorphonuclear cells infiltrate and breaks the mucosal and submucosal layers were still visible (Fig. 3D).

In day 11, the mucosa epithelium was fully recovered, however, some spots of the transmural lesion were still visible and the accumulation of what appears to be fibrotic tissue, accumulated adjacent to muscularis mucosae (Fig. 3E). Finally, 14 days after intestinal inflammation, the colon architecture was recovered. Even though edema was still visible, the mucosa and submucosa were completely regenerated (Fig. 3F), showing a colon architecture similar to the healthy colon (Fig. 3A).

Fig. 4 shows changes in the thickness of the mucosa, muscularis mucosae, submucosa, and muscularis externa, determined by histopathological quantification. The mucosal layer increased in thickness

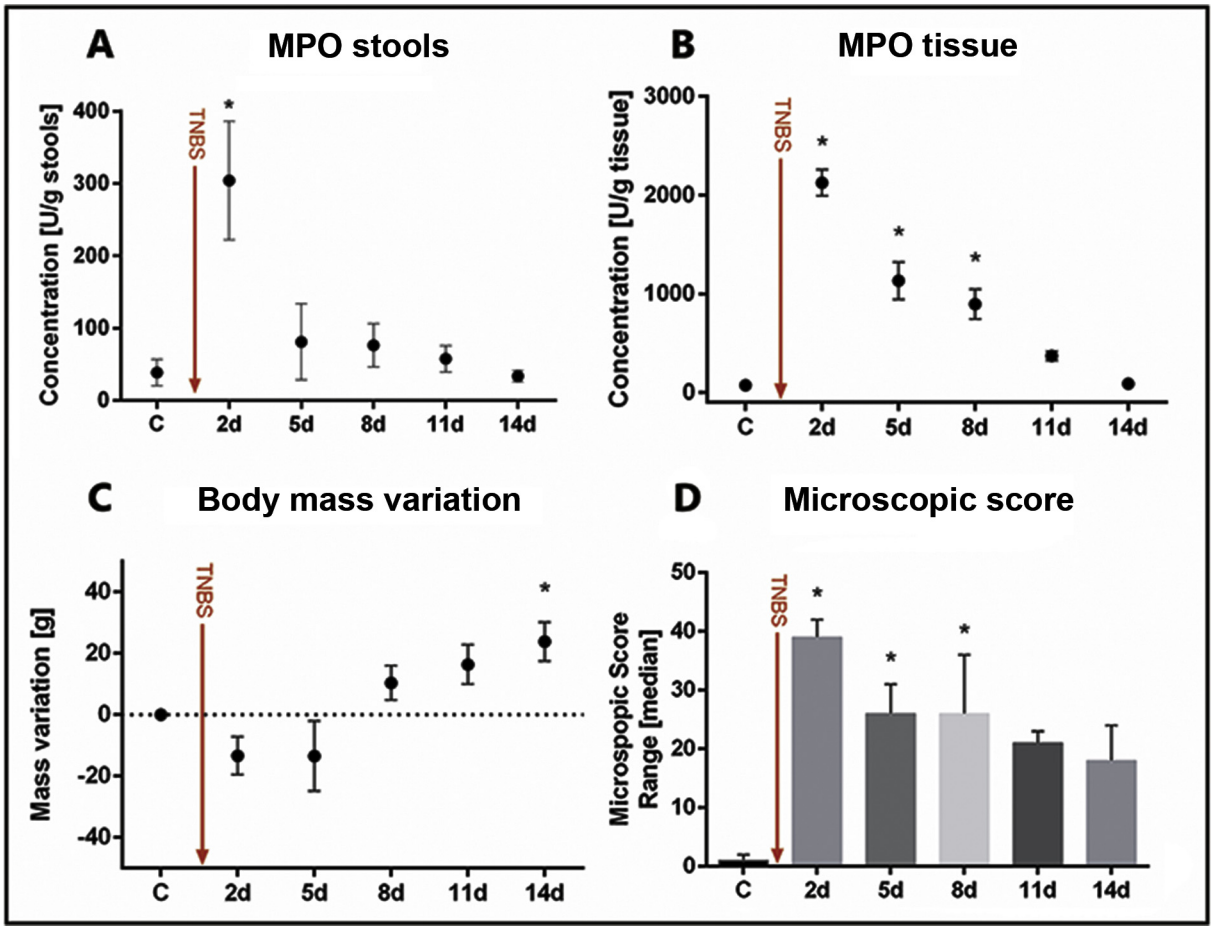

Fig. 2. Myeloperoxidase (MPO) activity in stools samples (A) and in colon tissue (B). Body mass variation (C) and semi-quantitative microscopic score (D) during the experiment. The vertical line indicates the moment of TNBS induction. *$p < 0.05$.

Table 1
Diarrhea occurrence in animals with intestinal inflammation induced by TNBS (Group 1).

Stage of inflammation	Diarrhea occurrence (%)
24h before TNNS induction (control)	0
2d	100[a]
5d	77,78[a]
8d	11,11
11d	0
14d	0

Control: before induction; 2d, 5d, 8d, 11d and 14d: 2, 5, 8, 11 and 14 days after induction.
[a] $0 < 0.5$.

2 days after induction, which lasted until the end of the experiment (Fig. 4B). The thickness of the submucosa (Fig. 4D) and muscularis externa (Fig. 4E) also were increased at day 2 and gradually decreased in the following assessments (5, 8, 11 and 14 days). The thickness of the muscular mucosa presented an increase only at day 5, followed by a tendency to decrease (Fig. 4C). The ulcerative area (Fig. 4F), exhibited the highest values at day 2 and beginning to decrease at day 5 and 8. Eleven days after induction, no ulceration was evident in the images. These data showed that excepting to mucosa, there was a time-dependent reduction of the muscularis mucosae, submucosa and muscularis externa thickness, also indicating different degree of the inflammatory process.

Fig. 5 shows a 10-min signal excerpt that exemplifies the mechanical activity of each acquisition moments (Control, 2, 5, 8, 11 and 14 days after induction). Gradual morphological differences were evident between all moments, especially 5 days after induction. Each signal shows the respective spectrum of frequencies, indicating the predominant frequencies that were contained in the signal (peaks). Throughout all of the measurements, we noticed the displacement of two prominent peaks, denoting a frequency change on RPMC and RPR activities, which probably was triggered by the inflammatory process.

Fig. 6 shows dominant frequencies variation for mechanical (RPMC and RPR) activities. The RPMCs frequency increased at 5 and 8 days after induction, returning to control values at 11 and 14 days after induction. We noticed a distinct behavior of the RPRs, in which their frequency decreased at 5 days after induction and remained statistically lower until 14 days after induction.

4. Discussion

In the present study, we performed an unprecedented characterization of colonic contractility and its response to the different degrees of intestinal inflammation induced by TNBS in rats, evaluated sequentially in the same animal. This was possible because the inflammatory process was monitored in the stool. Our results demonstrated some transient and permanent changes in colon contraction. This profile was related to histological parameters (thickness, microscopic score), clinical symptoms (body mass loss, diarrhea occurrence), tissue and fecal MPO activity.

We observed that effects of intestinal inflammation induced by TNBS were not restricted to the mucosa, but also extended to the submucosa and muscularis externa, thus potentially influencing neurotransmission and muscle contractility [27]. In addition to

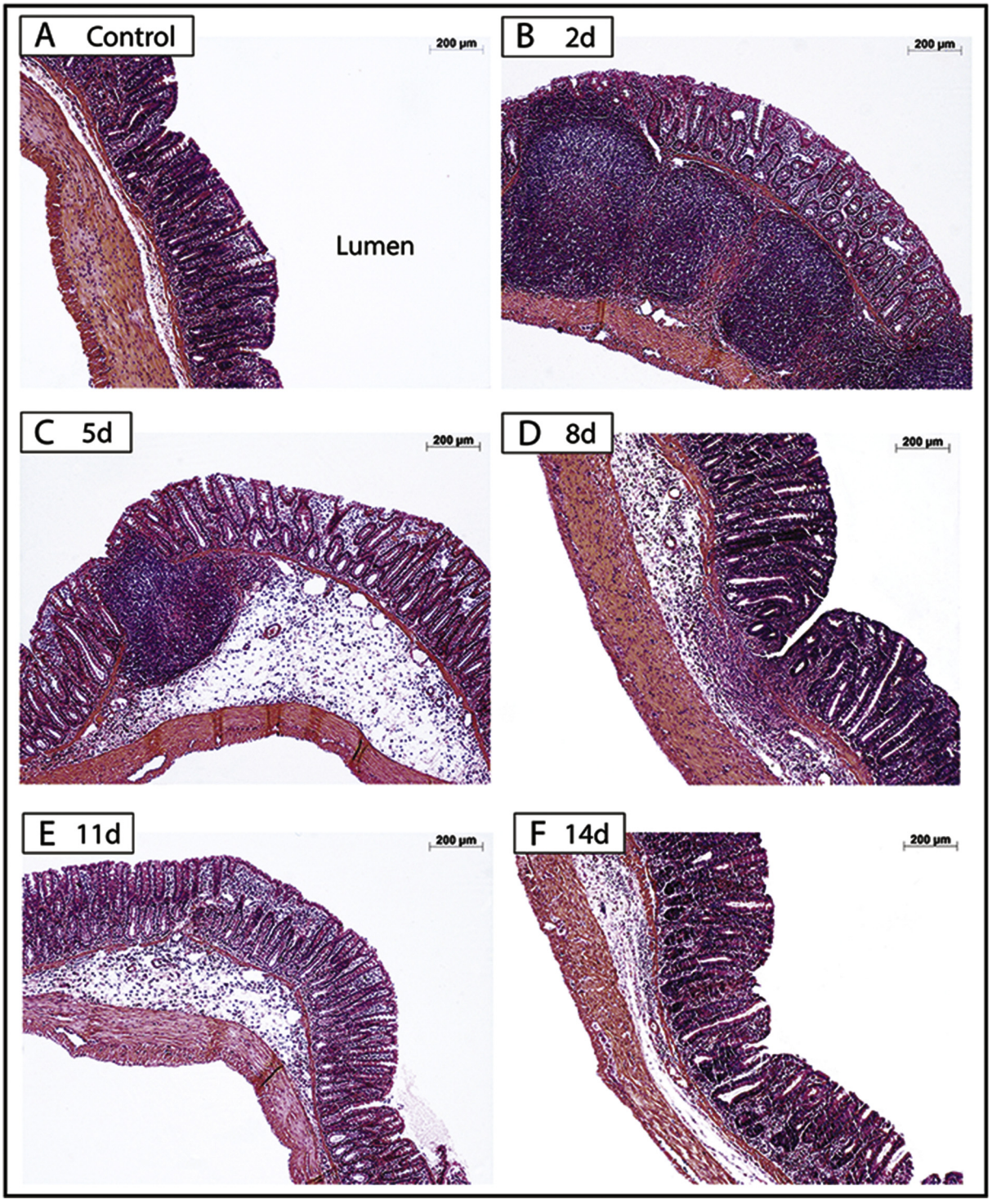

Fig. 3. Micrographs of colon tissue sample during the inflammatory process: (A) control; (B) two days after induction; (C) five days after induction; (D) eight days after induction; (E) eleven days after induction and (F) fourteen days after induction.

morphological changes, the profile and frequency of RPMCs and RPRs indicated abnormalities in motility throughout the experiment. The RPMCs transiently increased at 5 and 8 days after induction and then returned to control levels at 11 days after induction. Indeed, RPRs frequency decreased after induction of the inflammation process and was not restored until the end of the experiment. The main controllers of colonic motor activity involve the properties of smooth muscles, intrinsic nerves in the enteric nervous system, extrinsic nerves in the sympathetic and parasympathetic nervous systems, and chemicals that are released in the body or the circulation [28]. Although histological analyses are not appropriate for cell and nerve evaluation, due to the different damage observed and discussed, possibly the Interstitial Cells of Cajal (ICCs) and intrinsic nerves were damaged. Two days after induction, we noticed a mononuclear and polymorphonuclear cells infiltration in the submucosal and muscularis externa layers. In addition, edema markedly appeared in the submucosal layer, with similar increase of the muscularis mucosae, submucosa and muscularis externa layers at 2, 5 and 8 days after TNBS induction. Together, these effects of

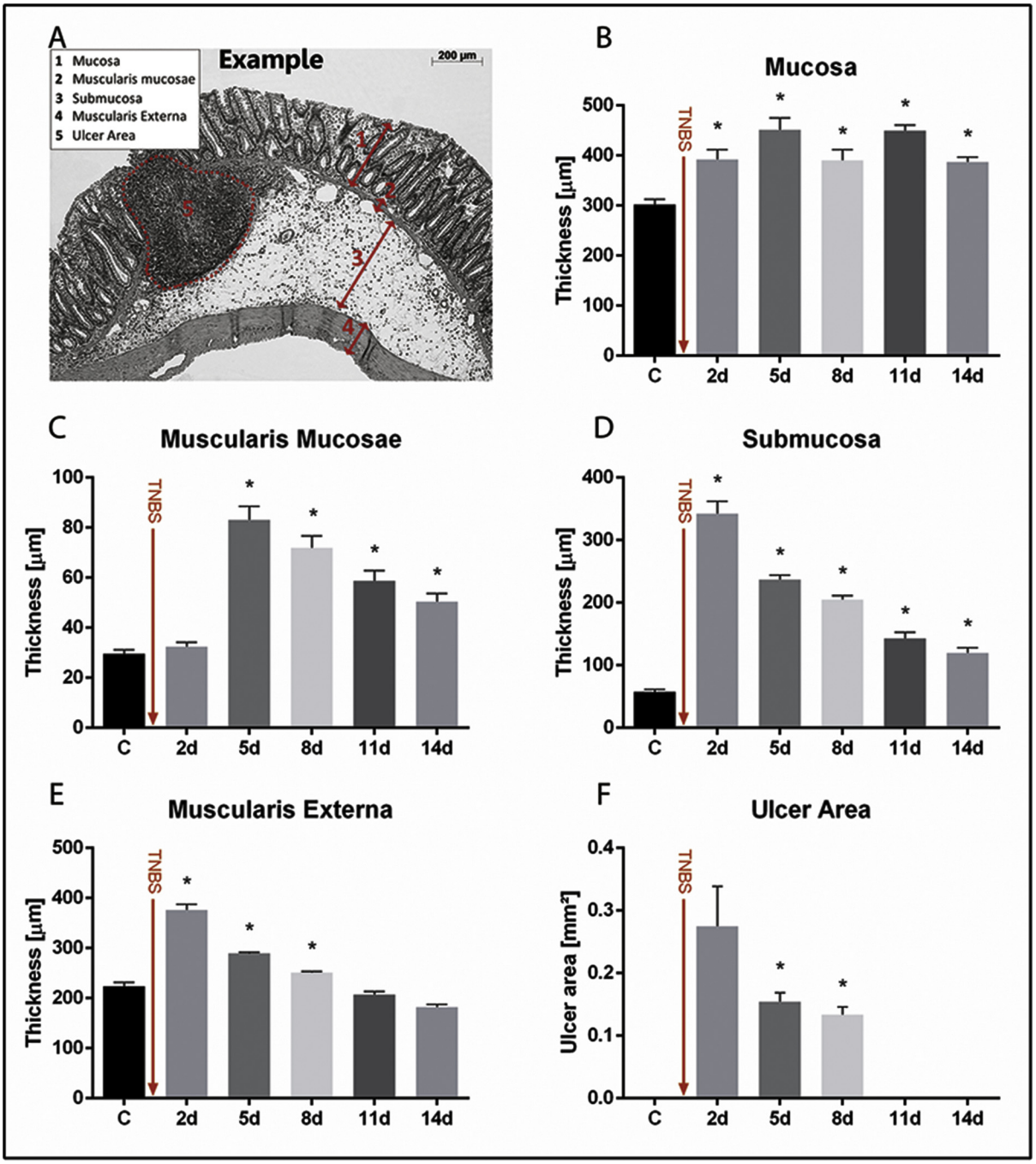

Fig. 4. Morphological changes of colon tissue layers and ulcer area during the inflammation process. (A) An illustrative example of layers identification; (B) mucosa; (C) muscularis mucosae; (D) submucosa; (E) muscularis externa; (F) ulcer area. The vertical line indicates the moment of TNBS induction. $^{*}p < 0.05$.

TNBS on the gut layers were directly related to changes in colon contractility.

Numerous studies indicated that intestinal inflammation promotes fibrosis in colonic tissue in several animal models, including TNBS model [29,30]. Although fibrosis was not measured, it is possible to infer that the fibrosis found in the mucosa and submucosa layers, at the recovery phase of intestinal inflammation, may be related to the frequency variation of RPR contraction. In this sense, this fibrosis process may have compromised the intrinsic nervous system (and/or ICCs) leading to irreversible damage [31].

Fig. 4 allows comparisons of the condition of the all layers thickness. In terms of the depth of the lesion, we observed that the muscularis externa layer was less damaged than the inner layers (muscularis mucosae, submucosa and mucosa). Even 14 days after inflammation induction, the mucosa and submucosal were not yet fully restored, suggesting that the damage was more severe when compared with the muscularis externa layer.

In this way, RPR activity, that is associated with more internal layers, has suffered greater influence on tissue damage. When specific pacemaker cells are removed or suffer irreversible damage, other ICCs that are nearby become dominant, provoking contraction frequency change permanently [15,33]. On the other hand, the RPMC, which is associated with the less damaged layers (external), underwent temporary changes.

The episodes of diarrhea at days 2, 5 and 8 may also be associated with the transient changes in the RPCM frequency. Diarrhea is

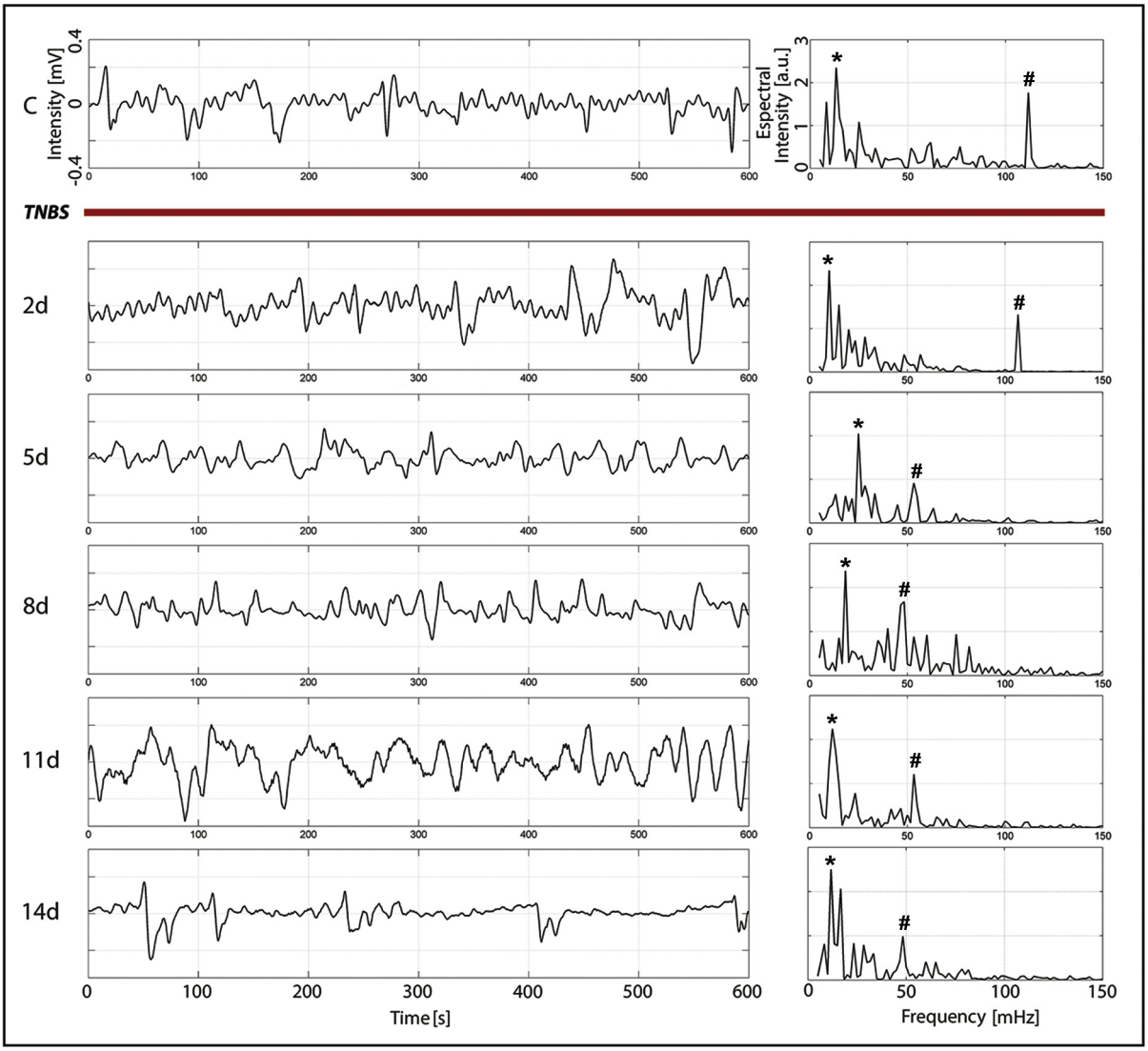

Fig. 5. Mechanical signals of all monitored stages and respective frequency spectra. The horizontal line indicates the moment of TNBS induction. * indicates RPMC frequency and # indicates RPR frequency.

characterized as a body's defense mechanism that clears the contents of the GIT, and it is closely related to increased motility and gastrointestinal transit [32]. Our results showed that the occurrence of diarrheal stools completely ceased from day 11, just when the RCMP frequency values returned to control values.

Alterations at the cellular level could be mainly responsible for changes in the contractility of intestinal smooth muscles [34]. The results for the RPMC and RPR mechanical contractions were very interesting, in which both frequency patterns underwent a significant change at day 5, whereas the MPO peak occurred at day 2, concomitant with the highest inflammation level. This delay may be related to the time at which the chemical injury became structural in terms of mechanical reflex and to the previously adopted protocol of a 3 days interval.

Ex vivo studies of the isolated rat colon showed that the TNBS-induced inflammatory response is associated with different restoration modes of spontaneous rhythmicity and myogenic contractions, especially during the healing stage of inflammation [8]. Spontaneous rhythmicity dysfunction, that is related to RPRs, may restore more rapidly than myogenic contractility dysfunction, which is related to RPMCs. This apparent contradiction may be related to the fact that this study [8] was performed *ex vivo* (*i.e.*, outside the physiological environment), with no contact with nervous regulation. Here, we employed an alternative approach for MPO analysis that allows for continuous assessment of inflammation degree over time, in contrast to the gold standard biopsy study [17]. Although the time-dependent reduction in stools MPO activity is not statically significant, except for day 2, the measurement of MPO activity in feces is a good non-invasive marker for the peak of acute inflammation. Therefore, this protocol represents a powerful tool because it is possible to maintain the animals alive throughout the entire experiment.

Based on our results, it is possible to suggest that ACB technique represents an efficient approach to monitor the motor activity of the colon, providing a clear distinction between propulsion (RPMC) and mixing (RPR) activities, and its consequences after the injury. In addition, we speculate that the different changes of each activity may be related to different types of structural damage in the intestine tissues (*e.g.*, muscular layers, fibrosis, neural damage). These considerations may contribute to a better understanding and future studies of motor responses, symptoms and treatments of intestinal diseases such as IBD, inflammatory bowel syndrome, diarrhea, constipation, megacolon, and peristalsis.

In summary, we implemented a robust and adequate (specific) signal processing to quantify two ACB-measured colonic frequency patterns, and thus we performed a detailed temporal analysis of the consequences of TNBS-induced inflammation on colonic motility in

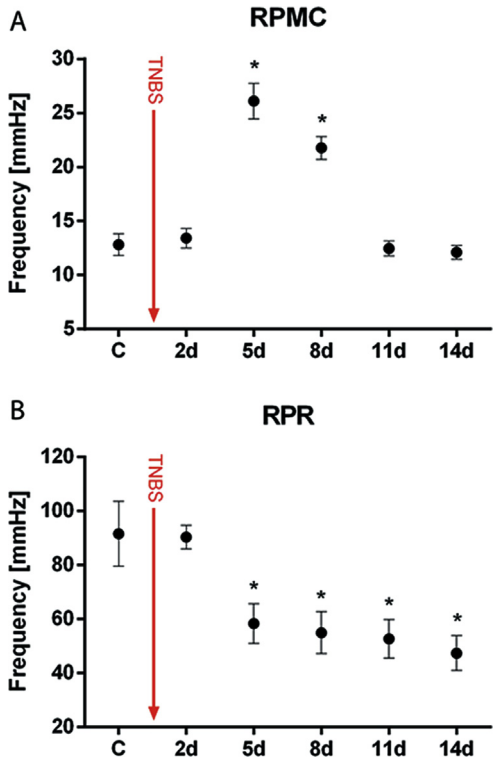

Fig. 6. Frequency variations in mechanical contractions ([A] RPMC, [B] RPR). The vertical line indicates the moment of TNBS induction. *$p < 0.05$.

rats. We utilized *in vivo* measurement technique and monitored biomarkers of inflammation in fecal samples for 14 days in the same animal. The observed changes in different contraction frequencies, through temporal analysis of the signals, revealed a clear relationship with the evolution of the lesions degree and contributing with a greater understanding of the motility, providing a powerful methodological tool.

Acknowledgments

Funding statement

The São Paulo Research Foundation – FAPESP (grant number 2013/09095-4 and 2015/14923-9) and National Council for Scientific and Technological Development (CNPq).

Declaration of competing interest

The authors declare that there are no conflicts of interest.

References

[1] L.C. Di Stasi, C.A. Costa, A. Witaicenis, Products for the treatment of inflammatory bowel disease: a patent review (2013 - 2014), Expert Opin. Ther. Pat. 25 (2015) 629–642.
[2] C. Fiocchi, Inflammatory bowel disease: etiology and pathogenesis, Gastroenterology 115 (1998) 182–205.
[3] G. Bouma, W. Strober, The immunological and genetic basis of inflammatory bowel disease, Nat. Rev. Immunol. 3 (2003) 521–533.
[4] G. Bassotti, E. Antonelli, V. Villanacci, M. Baldoni, M.P. Dore, Colonic motility in ulcerative colitis, United European Gastroenterol J 2 (2014) 457–462.
[5] C.O. Elson, R.B. Sartor, G.S. Tennyson, R.H. Riddell, Experimental models of inflammatory bowel disease, Gastroenterology 109 (1995) 1344–1367.
[6] G.P. Morris, P.L. Beck, M.S. Herridge, W.T. Depew, M.R. Szewczuk, J.L. Wallace, Hapten-induced model of chronic inflammation and ulceration in the rat colon, Gastroenterology 96 (1989) 795–803.
[7] P. Kiesler, I.J. Fuss, W. Strober, Experimental models of inflammatory bowel diseases, Cell. Mol. Gastroenterol. Hepatol. 1 (2015) 154–170.
[8] M. Kiyosue, M. Fujisawa, K. Kinoshita, M. Hori, H. Ozaki, Different susceptibilities of spontaneous rhythmicity and myogenic contractility to intestinal muscularis inflammation in the hapten-induced colitis, Neurogastroenterol. Motil. 18 (2006) 1019–1030.
[9] E. Antoniou, G.A. Margonis, A. Angelou, et al., The TNBS-induced colitis animal model: an overview, Ann. Med. Surg. 11 (2016) 9–15.
[10] V. Annese, G. Bassotti, G. Napolitano, P. Usai, A. Andriulli, G. Vantrappen, Gastrointestinal motility disorders in patients with inactive Crohn's disease, Scand. J. Gastroenterol. 32 (1997) 1107–1117.
[11] S.N. Reddy, S.M. Collins, E.E. Daniel, Frequency analysis of gut EMG, Crit. Rev. Biomed. Eng. 15 (1987) 95–116.
[12] Y. Sang Jin, K. Jie Hyun, S. Dae Hoon, L. Young Ho, P. Hyo Jin, L. Sang In, Original articles: changes in colonic transit and contractility of muscle according to the time course in TNBS-induced colitis, Korean J. Neurogastroenterol. Motil. 14 (2008) 122–128.
[13] J.D. Huizinga, II. Gastric motility: lessons from mutant mice on slow waves and innervation, Am. J. Physiol. Gastrointest. Liver Physiol. 281 (2001) G1129–G1134.
[14] C. Bossone, J.M. Hosseini, V. Pineiro-Carrero, T. Shea-Donohue, Alterations in spontaneous contractions in vitro after repeated inflammation of rat distal colon, Am. J. Physiol. Gastrointest. Liver Physiol. 280 (2001) G949–G957.
[15] M.F. Calabresi, C.C. Quini, J.F. Matos, et al., Alternate current biosusceptometry for the assessment of gastric motility after proximal gastrectomy in rats: a feasibility study, Neurogastroenterol. Motil. 27 (2015) 1613–1620.
[16] C.C. Quini, M.F. Americo, L.A. Cora, et al., Employment of a noninvasive magnetic method for evaluation of gastrointestinal transit in rats, J. Biol. Eng. 6 (2012) 6.
[17] J.E. Krawisz, P. Sharon, W.F. Stenson, Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models, Gastroenterology 87 (1984) 1344–1350.
[18] D.R. Hansberry, K. Shah, P. Agarwal, N. Agarwal, Fecal myeloperoxidase as a biomarker for inflammatory bowel disease, Cureus 9 (2017) e1004.
[19] L.A. Cora, M.F. Americo, R.B. Oliveira, et al., Biomagnetic methods: technologies applied to pharmaceutical research, Pharm. Res. 28 (2011) 438–455.
[20] M.F. Americo, R.G. Marques, E.A. Zandona, et al., Validation of ACB in vitro and in vivo as a biomagnetic method for measuring stomach contraction, Neurogastroenterol. Motil. 22 (2010) 1340–1344 (e1374).
[21] M. Shubair, M.A. Oriowo, I. Khan, Expression of alternatively spliced variants of Na-Ca-exchanger-1 in experimental colitis: role in reduced colonic contractility, Mol. Cell. Biochem. 370 (2012) 15–21.
[22] S.J. Kim, J.H. Park, D.K. Song, et al., Alterations of colonic contractility in long-term diabetic rat model, J. Neurogastroenterol. Motil. 17 (2011) 372–380.
[23] N. Paskaranandavadivel, G. O'Grady, P. Du, L. KC, Comparison of filtering methods for extracellular gastric slow wave recordings, Neurogastroenterol. Motil. 25 (2013) 79–83.
[24] American Gastroenterological Association medical position statement: short bowel syndrome and intestinal transplantation, Gastroenterology 124 (2003) 1105–1110.
[25] B. Arribas, M.E. Rodriguez-Cabezas, D. Camuesco, et al., A probiotic strain of Escherichia coli, Nissle 1917, given orally exerts local and systemic anti-inflammatory effects in lipopolysaccharide-induced sepsis in mice, Br. J. Pharmacol. 157 (2009) 1024–1033.
[27] Y.B. Gurung, Y. Shimizu, T. Shiina, M.E. Mahmoud, S. Saito, T. Takewaki, Impairment and restoration of spontaneous contractile activity of longitudinal smooth muscles in the TNBS-inflamed hamster distal colon, Biomed. Res. 28 (2007) 301–308.
[28] L.R. Johnson, Gastrointestinal Physiology, (2014).
[29] F. Rieder, S. Kessler, M. Sans, C. Fiocchi, Animal models of intestinal fibrosis: new tools for the understanding of pathogenesis and therapy of human disease, Am. J. Physiol. Gastrointest. Liver Physiol. 303 (2012) G786–G801.
[30] A.A. te Velde, M.I. Verstege, D.W. Hommes, Critical appraisal of the current practice in murine TNBS-induced colitis, Inflamm. Bowel Dis. 12 (2006) 995–999.
[31] K.M. Sanders, S.M. Ward, S.D. Koh, Interstitial cells: regulators of smooth muscle function, Physiol. Rev. 94 (2014) 859–907.
[32] M. Camilleri, L. Buéno, V. Andresen, F. De Ponti, M.-G. Choi, A. Lembo, Pharmacologic, pharmacokinetic, and pharmacogenomic aspects of functional gastrointestinal disorders, Gastroenterology 150 (2016) 1319–1331.e1320.
[33] W.J. Lammers, B. Stephen, Origin and propagation of individual slow waves along the intact feline small intestine, Exp. Physiol. 93 (2008) 334–346.
[34] R.W. Wells, M.G. Blennerhassett, Persistent and selective effects of inflammation on smooth muscle cell contractility in rat colitis, Pflugers Arch. 448 (2004) 515–524.