Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 430–436

Journal of Nutritional Biochemistry

Oral administration of sodium butyrate attenuates inflammation and mucosal lesion in experimental acute ulcerative colitis☆

Erica L.M. Vieira[a], Alda J. Leonel[a], Alexandre P. Sad[a], Nathália R.M. Beltrão[a], Thaís F. Costa[a], Talita M.R. Ferreira[a], Ana C. Gomes-Santos[a], Ana M.C. Faria[a], Maria C.G. Peluzio[b], Denise C. Cara[c], Jacqueline I. Alvarez-Leite[a,*]

[a]Department of Biochemistry and Immunology, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, 30161-970 Brazil
[b]Department of Nutrition, Universidade Federal de Viçosa, Viçosa, 31270-910 Brazil
[c]Department of Morphology, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, 30161-970 Brazil

Received 10 August 2010; received in revised form 13 January 2011; accepted 19 January 2011

Abstract

Butyrate is a four-carbon short-chain fatty acid that improves colonic trophism. Although several studies have shown the benefits of butyrate enemas in ulcerative colitis (UC), studies using the oral route are rare in the literature. In the present study, we evaluated the effect of butyrate intake in the immune response associated to UC. For that, mice were fed control or butyrate (0.5% sodium butyrate) diets for 14 days. Acute UC was induced by dextran sulphate sodium (DSS, 2.5%), replacing drinking water. The results showed that, in UC animals, oral butyrate significantly improved trophism and reduced leukocyte (eosinophil and neutrophil) infiltration in the colon mucosa and improved the inflammatory profile (activated macrophage, B and T lymphocytes) in cecal lymph nodes. In the small intestine, although mucosa histology was similar among groups, DSS treatment reduced duodenal transforming growth factor-β, increased interleukin-10 concentrations and increased memory T lymphocytes and dendritic cells in Peyer's patches. Butyrate supplementation was able to revert these alterations. When cecal butyrate concentration was analyzed in cecal content, it was still higher in the healthy animals receiving butyrate than in the UC +butyrate and control groups. In conclusion, our results show that oral administration of sodium butyrate improves mucosa lesion and attenuates the inflammatory profile of intestinal mucosa, local draining lymph nodes and Peyer's patches of DSS-induced UC. Our results also highlight the potential use of butyrate supplements as adjuvant in UC treatment.

Keywords: Butyrate; Ulcerative colitis; Inflammation; Lipids

1. Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) whose etiology is still unclear. Various mechanisms have been proposed, including inappropriate inflammatory response to pathogens, autoimmunity, abnormal immune response to microbiota or dietary antigens, etc. Short-chain fatty acids (SCFAs) derived from the bacterial fermentation of unabsorbed carbohydrates play an important role in maintaining colon pH and osmolarity [1]. Nevertheless, low SCFA concentration and impaired oxidation were described in patients with severe UC, contributing to the metabolic alterations of colonic mucosa. Butyrate, the four-carbon SCFA, is especially important to colon integrity since it is the main fuel for colonocytes. In addition, several *in vivo* and *in vitro* studies have shown the anti-inflammatory and anticarcinogenic effects of butyrate [2,3].

Short-chain fatty acid and butyrate enemas have been administered to patients presenting distal UC with favorable results. Studies have shown that about 50% of UC patients reduced colon inflammation after local irrigation with butyrate and also that 50% of patients who did not respond to corticosteroid therapy improved histological and clinical symptoms after butyrate enemas [4–6]. Although those studies support the beneficial effect of butyrate treatment in UC patients, it is only administrated as enema or indirectly produced by a high fermentable fiber or butyrate-producing bacteria supplementation [3,7,8]. Oral administration of butyrate, nevertheless, is a route less explored since it is believed that butyrate would not reach the colon due to its rapid gastric and duodenal absorption. Few studies addressed the effect of oral administration of SCFA or butyrate either as single treatment or as adjuvant of conventional therapy for IBD studies [9–11]. However, in those studies, butyrate was given as enteric-coated tablets or as a formulation for release in the colon.

☆ This study was supported by grants from the National Council for Scientific and Technological Development (CNPq), Coordination of Improvement of Higher Level Personnel (CAPES) and Foundation for Research Support of Minas Gerais (FAPEMIG).

* Corresponding author. Laboratório de Aterosclerose e Bioquímica Nutricional, Departamento de Bioquímica e Imunologia ICB/UFMG. Caixa Postal 486-CEP 30161-970. Belo Horizonte, MG, Brasil.

E-mail address: jalvarezleite@gmail.com (J.I. Alvarez-Leite).

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2011.01.007

Therefore, the aim of the present work was to evaluate the efficacy of oral administration of butyrate on the acute UC induced by dextran sulphate sodium (DSS) in mice.

2. Methods

Forty C57BL/6 mice received for 14 days control (AIN-93G) [12] or butyrate (control diet+0.5% of sodium butyrate) diets. Sodium butyrate was prepared by addition of NaOH to butyric acid (Sigma, St. Louis, MO, USA), pH=7.4. Ulcerative colitis was induced by the oral intake of DSS, 2.5% in water, replacing drinking water. Animals were divided into four groups: control and butyrate groups received the respective diets without UC induction, and UC and UC+butyrate groups received the respective diets and DSS from the 7th to 14th experimental days. The animals were kept in collective cages in an environment with light/dark cycles of 12 h and free access to water or DSS solution. At the 15th day, all animals were anesthetized and sacrificed for blood and organ collection.

SCFA analyses were done in the cecal content. The extraction and analysis of these fatty acid were done as previously described [13]. The small intestine and colon were taken for histological analysis. The samples were fixed in 10% formalin [phosphate-buffered saline (PBS)], embedded in paraffin, cut into 3–5-μm thick sections and stained with hematoxylin–eosin. Morphometric analyses of crypts and villi were performed using an image analysis program (KS 300-Zeiss) running on an IBM computer.

For flow cytometry analysis, 100 μl of blood (collected with EDTA) was diluted (1:50) and mixed with 15 μl of monoclonal antibodies (BD, Becton Drive, NJ, USA). Combinations of antibodies included IgG2a-FITC/IgG2a-PE, CD4-FITC/CD8-PE, CD11b-FITC/CD11c-PE and CD19-PE. Preparations were fixed and analyzed in a FACScan flow cytometer (FACScan Becton Dickinson) using the CELLQuest software for acquisition and data analysis (Becton Dickinson, San José, CA, USA).

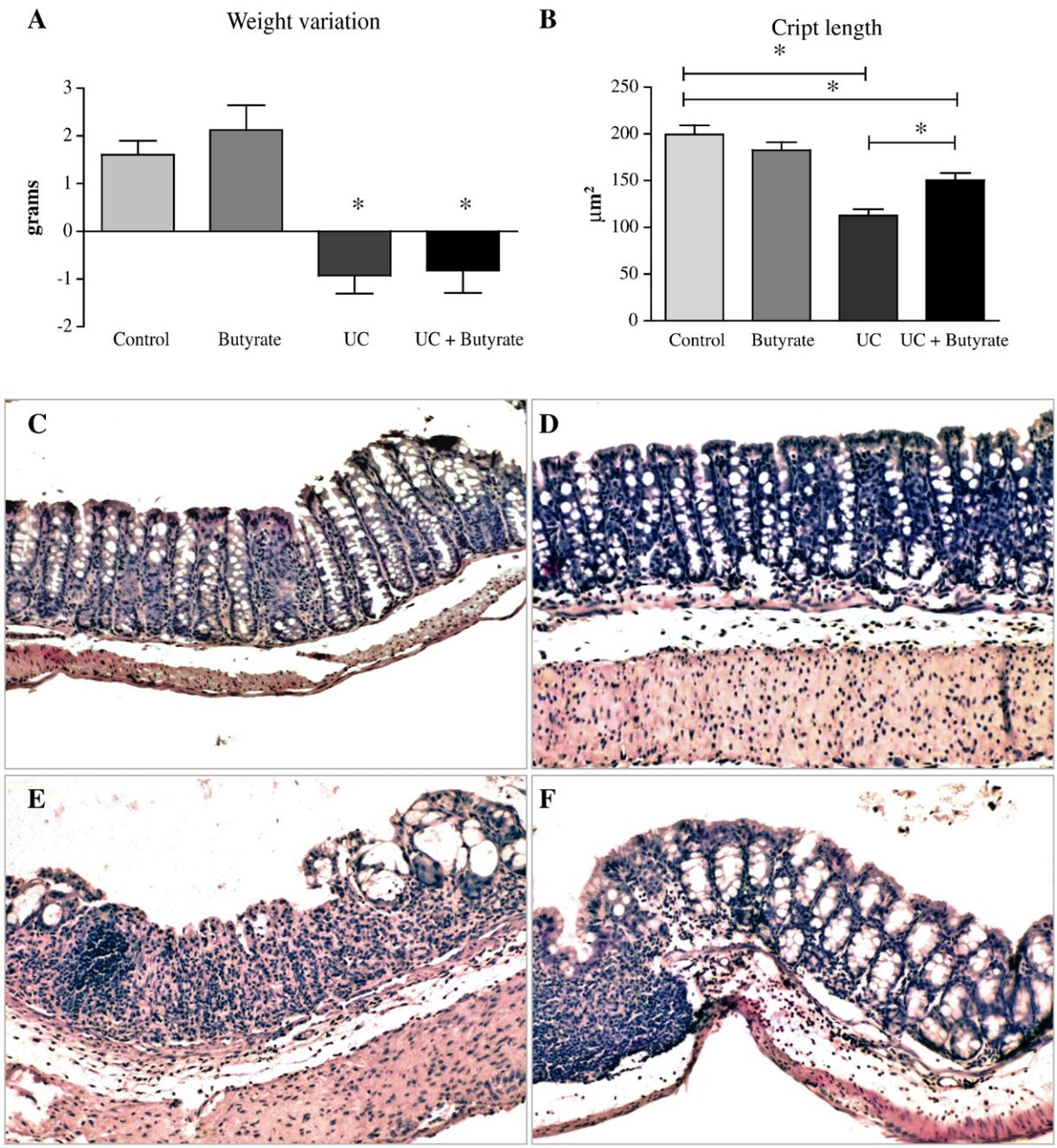

Fig. 1. Weight variation (A), crypt length (B) and histopathological aspect of colon mucosa of animals from the control group (C) receiving standard diet for 15 days, the butyrate group (D) receiving standard diet+0.5% of sodium butyrate for 15 days, the UC group (E) receiving standard diet for 15 days and DSS in the last 7 days, replacing drinking water, and the UC+butyrate group (F), receiving butyrate-rich diet for 15 days and DSS in the last 7 days.

Cell suspensions of spleen were prepared as previously described [14]. Cells were incubated for 30 min with the fluorochrome-conjugated monoclonal antibody of interest. Samples were washed, centrifuged, fixed and then analyzed in the same flow cytometer using CellQuest software for acquisition and data analysis. For each immunophenotyping, information about morphometric aspects of size and granularity and immunophenotypic features of 30,000 events were collected.

Activity of the enzymes myeloperoxidase (MPO), *N*-acetylglucosaminidase (NAG) and eosinophil peroxidase (EPO) was assessed in samples of small intestine and colon as previously described [15]. Samples were homogenized and centrifuged, and precipitates were used for quantification of enzyme activities. Precipitates were dissolved in HETAB 0.5% in phosphate buffer, and after homogenization and freezing/thawing sessions, suspensions were centrifuged and used for future determinations. For EPO quantification, 75 μl of supernatant was added to 75 μl of OPD (diluted in Tris-HCl and H_2O_2) and incubated at 37° C in the dark for 30 min. Reaction was stopped by adding 50 μl H_2SO_4 and then read at 492 nm. For MPO quantification, 25 μl of supernatant were added to 25 μl of 3,3′,5,5′-tetramethylbenzidine (TMB) in dimethyl sulfoxide (DMSO) and incubated at 37°C for 5 min. After that, 100 μl of H_2O_2 was added and incubated. Reaction was stopped by adding H_2SO_4 and read at 450 nm. For NAG quantification, precipitates were dissolved in Triton X-100 (0.1%) and centrifuged, and 100 μl of supernatant was added to 100 μl of *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine in citrate/phosphate. After incubation, 100 μl of glycine buffer was used to stop the reaction, which was then read at 400 nm. Results were expressed in arbitrary units (based on absorbance) by 100 mg of tissue.

Cells from lymph nodes and Peyer's patches were separated for the study of immune cell profile. Organs were removed and placed in tubes containing RPMI 1640 complete medium, homogenized and centrifuged at 1200 rpm for 5 min at 4°C. The supernatant was discarded, and the pellet was resuspended in PBS containing 0.5% bovine serum albumin and 0.01% azide. Ten microliters of PBS-diluted antibodies (1:100) was homogenized with 25 μl of cell suspension and incubated for 30 min at 4°C in the dark, washed and fixed (FACS FIX MACS). Data were acquired using FACSCalibur (BD Biosciences, San Jose, CA, USA) equipment and analyzed by Flowjo (Treestar USA) software.

For cytokine assay, the small intestines were removed from euthanized mice, separated into duodenum, jejunum and ileum and placed in an extract buffer solution (1 ml/g). Tissue was homogenized and centrifuged for 15 min at 600*g* at 4°C. Supernatants were collected for cytokine assay. Plates were coated with monoclonal antibodies overnight and washed, and supernatants were added and incubated overnight. After that, biotinylated antibodies are added and incubated for 1 h. Color reaction was developed with orthophenylenediamine and H_2O_2 substrate in sodium citrate buffer. Reaction was interrupted by the addition H_2SO_4. Absorbance was measured at 490 nm by an enzyme-linked immunosorbent assay reader (Bio-Rad Model 450 Microplate Reader).

Statistical analysis was performed using the GraphPad Prism 5.0 software (San Diego, CA, USA). The results were tested for outliers and normality using Shapiro–Wilk and box plot tests, respectively. Kruskal–Wallis test and Dunn's posttest were used in nonparametric analysis, while one-way analysis of variance (ANOVA) and Tukey's posttest were used in case of normal distribution. A significant difference was defined as $P \leq .05$.

3. Results

Weight and food intake were similar among the groups before the induction of UC (data not shown). As expected, DSS treatment induced weight loss in UC groups, regardless of butyrate supplementation (Fig. 1).

In UC mice, several manifestations of the disease were detected such as diarrhea, fecal blood, weight loss, mucosa injury and ulcerations. Reductions of crypt length, associated to a large influx of inflammatory cell, were also seen in DSS-treated animals (Fig. 1). Butyrate supplementation was able to improve mucosa inflammation, diarrhea and crypt length when compared to the UC group.

Dextran sulphate sodium treatment triggered an increase of 15 times in neutrophils and 6 times in eosinophil infiltration to the lesion area (Fig. 2). Butyrate intake reverted leukocyte infiltration and reduced by 75% the mucosa eosinophil concentration compared to the control and butyrate groups. Macrophage infiltration, on the other hand, was similar in all groups compared to the control one.

Although DSS caused a severe colonic lesion, it was not importantly reflected in the blood and spleen cells (Table 1). In the blood, the butyrate+UC group presented a lymphocyte frequency intermediate between the control and UC groups. In the spleen, although neutrophils contributed only a minor proportion of total cells, it was higher in the UC group compared to the control animals. Butyrate failed in preventing such increase. When T and B

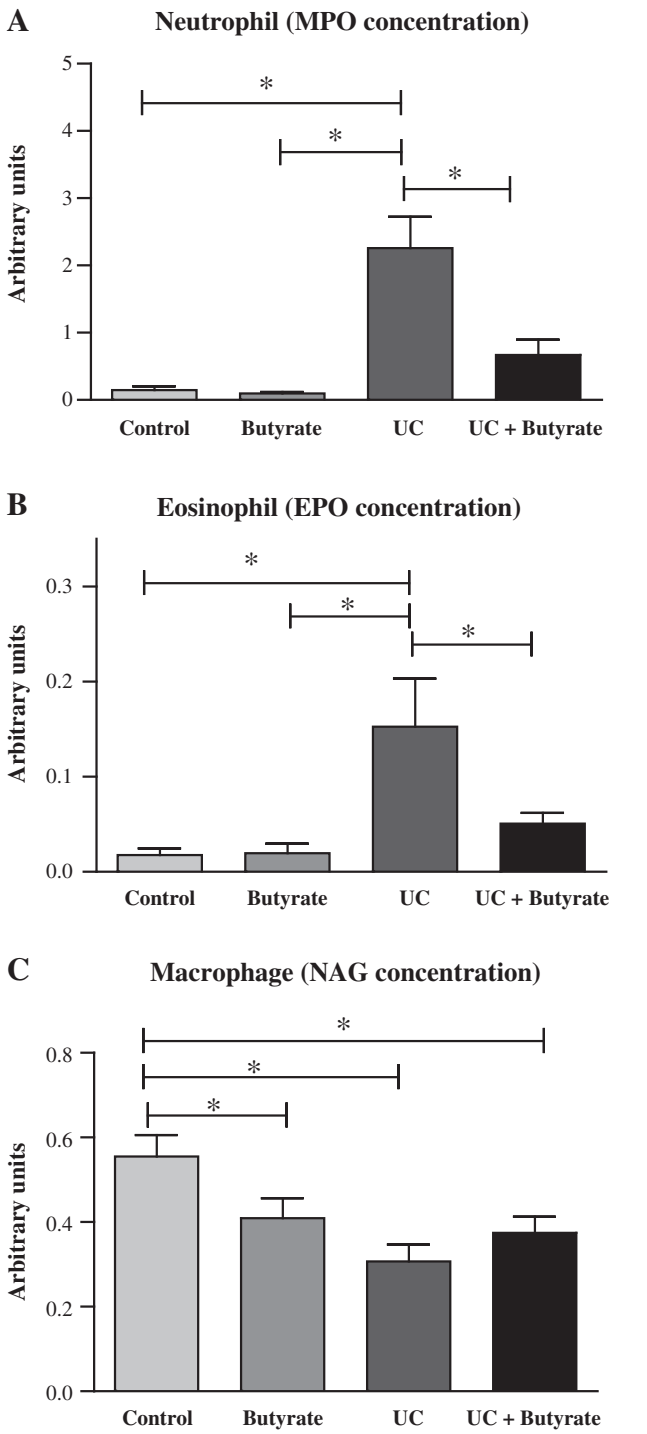

Fig. 2. Activity of (A) neutrophil MPO, (B) EPO and (C) macrophage NAG. Animals received either standard diet (control and UC group) or butyrate-rich diet (control+butyrate or UC+butyrate) throughout the 15-day time period. The control group received plain drinking water throughout, while the UC group received plain water from days 1 to 8 and water containing 2.5% DSS from days 9 to 15.

lymphocytes were analyzed (Table 2), B cells were increased in the spleen of both UC groups. Butyrate was not able to reduce B cells in both the blood and spleen (Table 2).

To study the immune response in the gut-associated lymphoid organs, we analyzed lymphocytes, macrophages and dendritic cells in mesenteric and cecal lymph nodes (CLNs) as well as in Peyer' patches (Fig. 3). In CLN, DSS treatment increased the percentage of mature

Table 1
Frequency of major immune cells on the blood, spleen and mesenteric lymph nodes of control or UC mice treated with saline or sodium butyrate for 15 days

Group	Lymphocyte	Monocyte	Dendritic cell	Neutrophil	Eosinophil
Blood					
Control	75.2 [a] (2.0)	4.5 (0.6)	5.3 (3.1)	7.0 [a] (0.6)	0.8 (0.2)
Butyrate	74.5 [a] (2.1)	5.6 (0.8)	6.1 (3.0)	7.7 [a] (0.5)	0.7 (0.2)
UC	64.2 [b] (3.8)	6.9 (0.6)	5.7 (2.9)	13.4 [b] (2.1)	0.5 (0.1)
UC+butyrate	70.2 [ab] (2.3)	4.2 (0.6)	3.4 (1.3)	13.9 [b] (1.9)	0.6 (0.1)
Spleen					
Control	74.5 (1.5)	6.4 (0.5)	3.3 (0.4)	1.9 [a] (0.1)	0.19 (0.05)
Butyrate	74.6 (0.5)	6.6 (0.3)	3.5 (0.2)	2.2 [ac] (0.2)	0.14 (0.03)
UC	72.4 (0.9)	6.2 (0.4)	3.8 (0.3)	3.0 [bc] (0.2)	0.12 (0.03)
UC+butyrate	71.7 (1.8)	6.4 (0.5)	3.3 (0.4)	3.7 [b] (0.1)	0.19 (0.05)

Different letters in a same column=statistical difference ($P<.05$), ANOVA and Newman–Keuls multiple comparison test.

antibody producing B cells (CD19+CD21+) and macrophages (CD11c−CD11b+) but reduced the frequency of activated (CD69+) CD4+ T cells. Mice from the UC+butyrate group improved these parameters, returning the frequencies closer to control levels.

Although DSS induced histological alterations only in the colon, differences in the lymphoid tissue could be detected in the small intestine of both UC groups. The percentage of B.1 (CD19+CD5+) cells, macrophages (CD11b+CD11c−) and memory (CD44+) CD4+ T cells in mesenteric lymph nodes and in Peyer's patches was also influenced by DSS. Nonetheless, butyrate treatment was able to normalize these parameters in Peyer's patches but not in mesenteric lymph nodes. The pattern of cytokines in the small intestine was also improved by butyrate (Fig. 4). Transforming growth factor (TGF)-β, which was reduced in the DSS group, tended to increase after butyrate administration. Interleukin (IL)-10 concentration, on the other hand, which was higher in duodenum of animals from the DSS group, decreased to control levels after butyrate treatment.

Short-chain fatty acids are believed to be quickly absorbed in the stomach and proximal intestine after oral administration, justifying their use only as enema in colon diseases. To verify if oral butyrate reaches the colon, we measured SCFAs in the cecum content. Interestingly, concentrations of acetate and propionate were more than twice higher in UC animals. This increase was prevented by butyrate supplementation (Fig. 5A and B). When butyrate concentration was analyzed, it was three times higher in the butyrate group compared to the control one (Fig. 5C). However, in the UC+butyrate group, butyrate concentration was reduced to the levels seen in nonsupplemented animals.

4. Discussion

Although several studies have shown the benefits of butyrate enemas in UC, studies using the oral route are rare in the literature [2,5,7,8]. Our results confirmed the trophic effect of this fatty acid on colon mucosa seen by others using rectal administration [7,8,16,17]. Moreover, we showed that its beneficial action on UC can also be obtained by oral route.

Since innate immunity has an important role on the colitis development, we evaluated the effects of butyrate on the immune cell profile in different organs. Oral butyrate reduced colonic inflammation as demonstrated by the improvement of colonic leukocyte profile in UC+butyrate animals. It is well known that neutrophil migration is responsible for the extensive colon damage as well as the release of toxic components and free radicals [18,19]. Butyrate treatment was able to reduce neutrophil in acute UC, confirming its anti-inflammatory effect. Our results are in agreement with other studies showing the effect of rectal administration of butyrate in protecting colon mucosa and reducing neutrophil infiltration after DSS or ischemia reperfusion injuries [8,17,20].

Eosinophil infiltration was also influenced by both DSS and butyrate treatment. It has been proposed that eosinophil acts as an immune "sentinel" due to its rapid migration to the inflammatory site. The influx of eosinophil in colon of IBD patients was already described [21–23], and its detection, even in small quantities, has been associated to adverse clinical consequences such as weight loss, malabsorption and reduction of crypt length [24]. Eosinophil triggers tissue damage due to the release of lipid mediators such as platelet activating factor, leukotrienes and toxic granule protein [24,25]. It has been suggested that eosinophils also act as antigen-presenting cells that stimulate T cell proliferation and activation [26]. Although the effect of butyrate in eosinophil differentiation has been already demonstrated [27], more studies are necessary to understand the role of butyrate in reducing eosinophil infiltration in our model of IBD.

Butyrate was described to influence lymphocyte activation *in vitro* [28,29]. In our experiments, blood and splenic lymphocytes were not importantly altered by the disease or by butyrate treatment. It is likely that the short course of DSS administration (7 days) and low DSS concentration (2.5%) triggered an acute and localized form of inflammatory disease that was not reflected in extra-intestinal environment.

Cecal lymph node is an important regional lymphoid organ for the colonic mucosa. Inflammatory bowel diseases are associated to increased number of activated B lymphocytes in the gut lymphoid tissue followed by an increase of inflammatory IgG [30] that has a deleterious effect for colonic mucosa. Our results are in agreement with previous reports showing that activated B cells rise in CLNs of UC mice. It is also interesting that butyrate inhibited macrophage migration to CLN since these cells play a major role in the pathogenesis of IBD. Recent evidences indicate that DSS-induced UC triggers the production of colonic eotaxin (CCL11) derived from activated macrophage, suggesting that those cells represent a source of chemotactic factors that attract and activate eosinophils to the colonic mucosa in UC [31]. These results are in concert with our finding of a higher eosinophil migration to colon associated to the increase levels of macrophage in CLNs of UC mice. Once again, butyrate acted as an anti-inflammatory agent, improving the profile of these immune responses.

In our acute model, recently activated T cells were reduced in CLN of UC mice. Activated CD4+ T cells are known as important players in IBD [32]. Although this result seems to be contradictory in the face of the proinflammatory profile of UC, it is likely that colitis associates to a strong T cells activation that could culminate in their apoptosis in the draining lymph node (CLN). It could explain the

Table 2
Frequency of major subtypes of lymphocytes, spleen and mesenteric lymph nodes of control or UC mice treated with saline or sodium butyrate for 15 days

Group	CD4+T cells	CD8+ T cells	CD19+ B cells
Blood			
Control	22.6 (0.7)	15.4 (0.4)	49.5[a] (1.4)
Butyrate	22.3 (0.9)	15.1 (0.6)	51.1 [ab] (1.8)
UC	20.7 (1.4)	14.1 (1.0)	55.9 [b] (1.8)
UC+butyrate	20.8 (1.1)	16.7 (1.5)	54.5 [ab] (1.7)
Spleen			
Control	10.5 (0.7)	17.5 [a] (1.2)	33.2 [a] (2.8)
Butyrate	9.7 (0.8)	17.2 [a] (1.0)	38.3 [ab] (2.3)
UC	9.9 (0.9)	15.6 [b] (0.8)	39.9 [b] (2.2)
UC+butyrate	9.2 (1.0)	15.8 [b] (0.7)	45.4 [b] (2.7)

Different letters in a same column=statistical difference ($P<.05$), ANOVA and Newman–Keuls multiple comparison test, except *Dunnett's multiple comparison test.

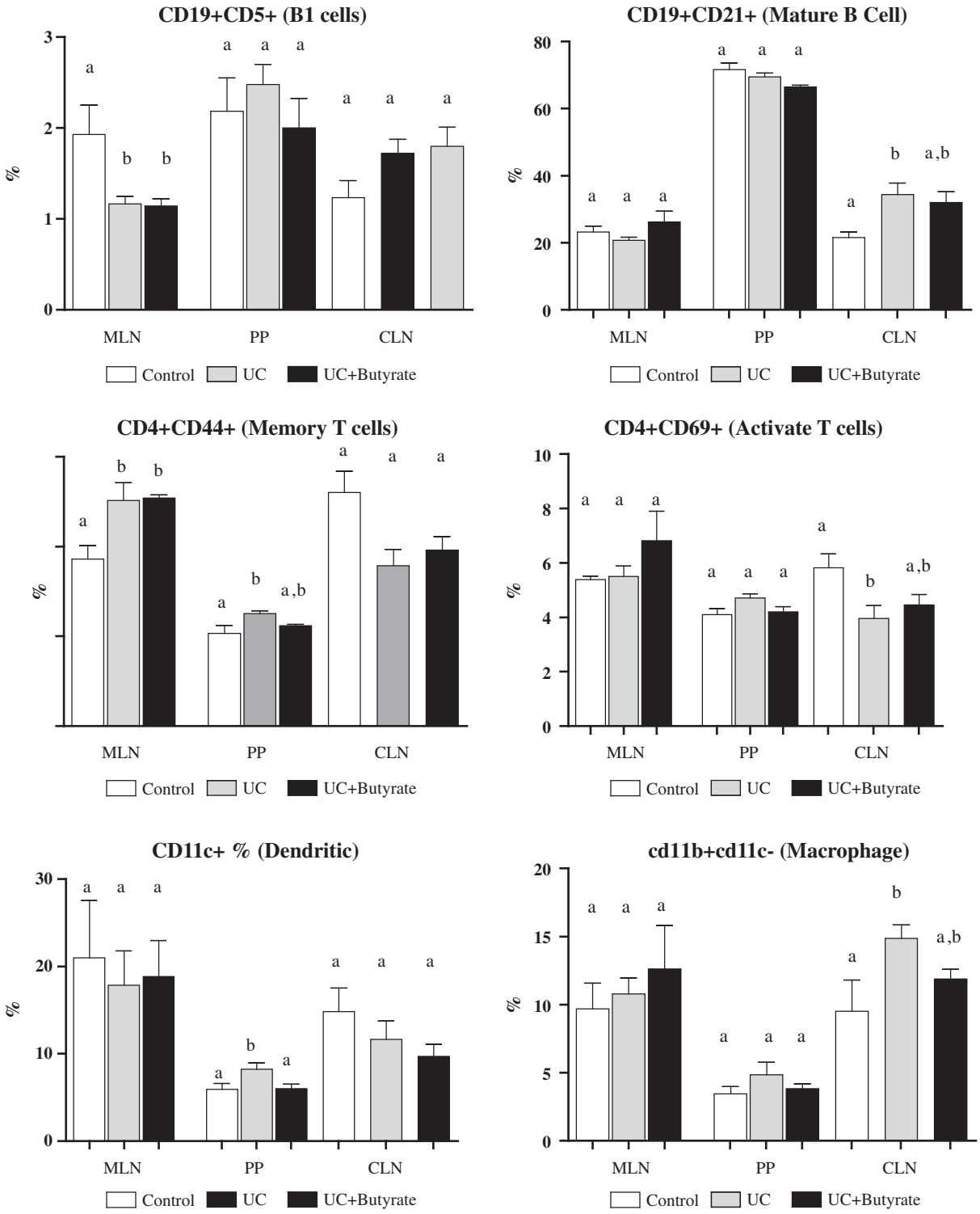

Fig. 3. Flow cytometry of lymphocyte subtypes in mesenteric lymph node (MLN), Peyer's patches (PP) and CLN. Animals received either standard diet (control and UC group) or butyrate-rich diet (control+butyrate or UC+butyrate) throughout the 15-day time period. The control group received plain drinking water throughout, while the UC group received plain water from days 1 to 8 and water containing 2.5% DSS from days 9 to 15.

reduction of activated T cells in the UC group compared to the control (basal) levels. Butyrate was also able to return activated T cells to the basal levels.

Duodenum is the region that concentrates the highest frequency of lymphocytes in the gut mucosa [33]. Interestingly, DSS-induced colitis influenced the production of anti-inflammatory cytokines in the duodenum, reducing TGF-β and increasing IL-10 levels. Since these cytokines exert crucial roles in the intestinal homeostasis, it is possible that the rise of IL-10 represents a compensatory mechanism. It is noteworthy that butyrate treatment prevents TGF-β and IL-10 alterations, suggesting its ability to maintain the cytokine balance in normal levels.

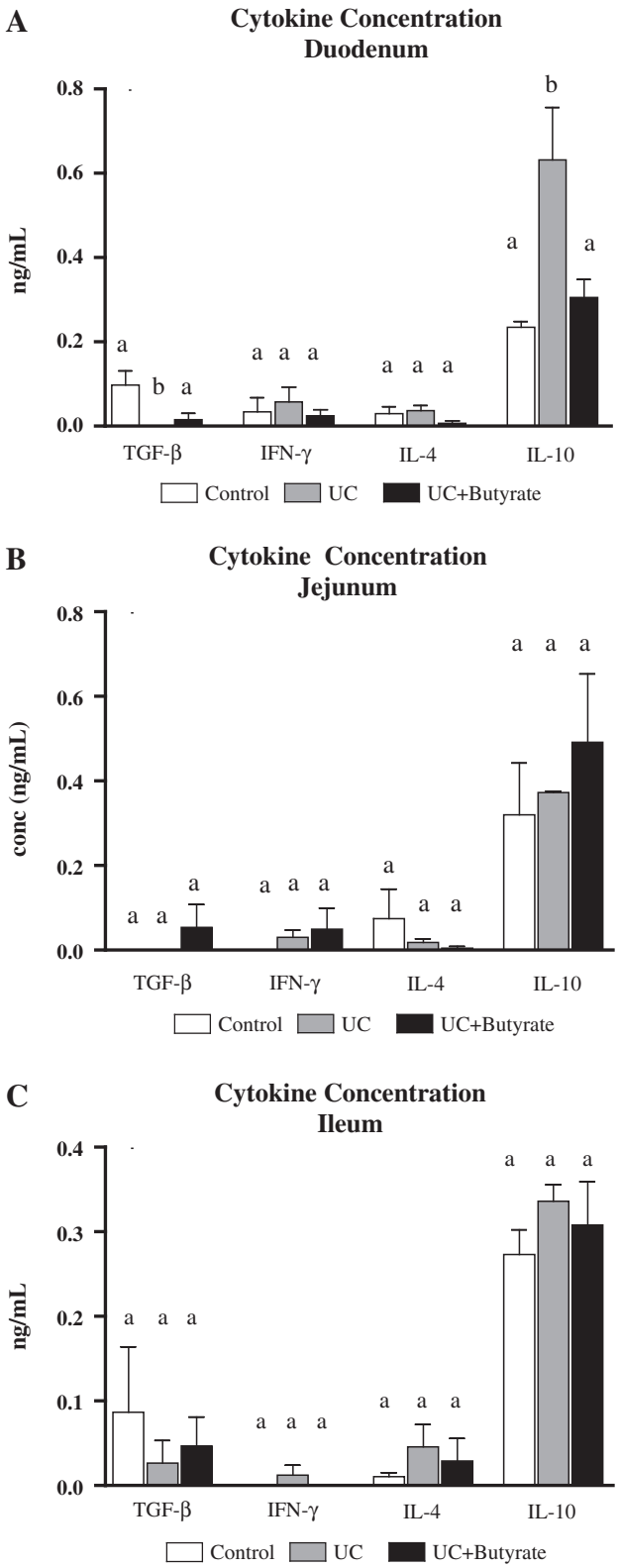

Fig. 4. Cytokine concentration on duodenum (A), jejunum (B) and ileum (C). Animals received either standard diet (control and UC group) or butyrate-rich diet (control+ butyrate or UC+butyrate) throughout the 15-day time period. The control group received plain drinking water throughout, while the UC group received plain water from days 1 to 8 and water containing 2.5% DSS from days 9 to 15.

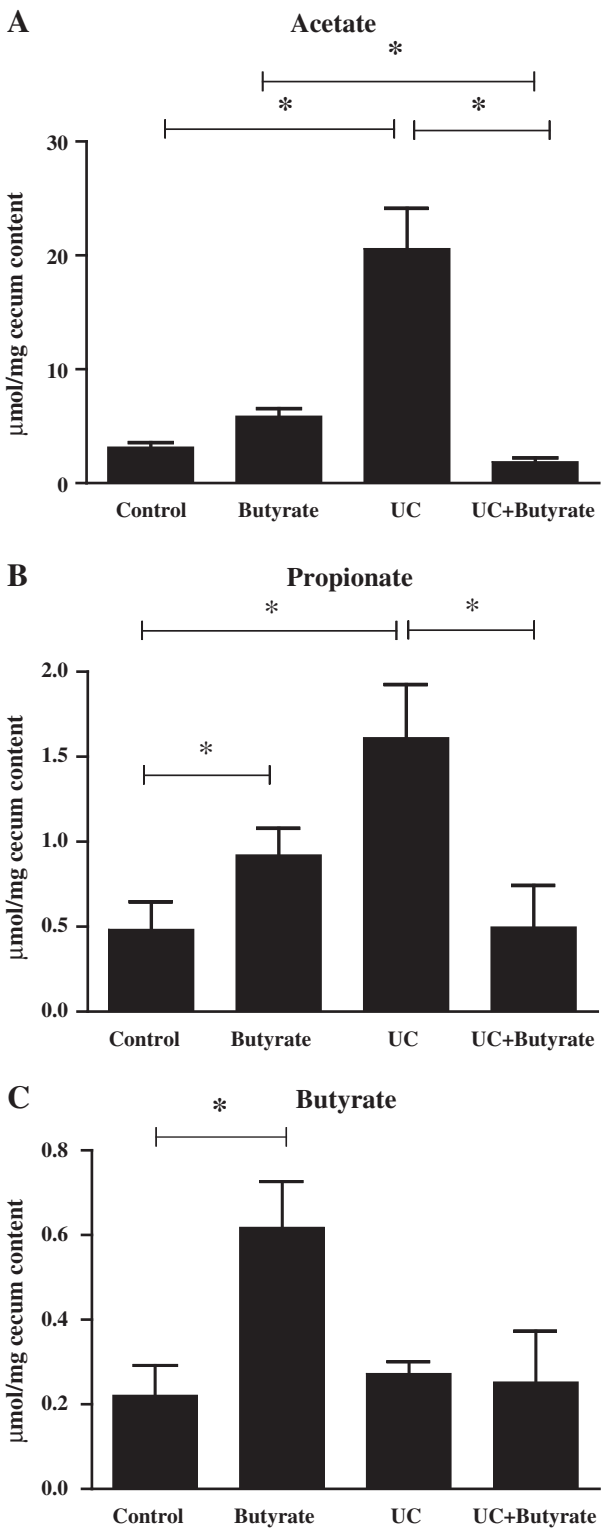

Fig. 5. Acetate (A), propionate (B) and butyrate (C) concentration in cecum. All groups received either standard diet (control and UC group) or butyrate-rich diet (control+ butyrate or UC+butyrate) throughout the 15-day time period The control group received plain drinking water throughout, while the UC group received plain water from days 1 to 8 and water containing 2.5% DSS from days 9 to 15.

Our results also showed that the concentration of butyrate entering in the colon was still higher in the butyrate group after oral administration, suggesting that this fatty acid was not totally absorbed in small intestine. Interestingly, butyrate concentration was reduced in the UC+butyrate animals, suggesting that the improvement of mucosa damage was associated to its increased metabolism or absorption in the small intestine. These data suggest that butyrate could exert direct effects in the colon after oral supplementation.

Previous studies described the beneficial effect of SCFA on intestinal mucosa when given by parenteral route [34,35]. In those studies, the intravenous infusion of SCFA was effective in inhibiting mucosal atrophy associated to parenteral nutrition or intestinal resection. It was suggested that hepatic metabolism of butyrate may produce substances such as glutamine and acetoacetate, important fuels for the intestinal metabolism.

Altogether, those data suggest that butyrate or butyrate-derived substances contribute to the UC remission by two ways: directly as the main fuel for the colonic mucosa or indirectly by the hepatic production of glutamine and acetoacetate that are also trophic agents for the intestine. Moreover, we believe that the effect of butyrate on inflammation and inflammatory cells can be secondary to the improvement of mucosa integrity that by itself reduces inflammation. Alternatively, the circulating levels of butyrate and butyrate-derived glutamine could also be sufficiently high as to affect immune cells since both are recognized immune modulators [28,36]. The exact mechanism of the beneficial effects of oral butyrate is still unexplored and is beyond the purpose of the present study.

In conclusion, we showed that oral administration of sodium butyrate improves mucosa lesion and attenuates inflammatory profile of intestinal mucosa and local lymph nodes in a model of DSS-induced colitis. Our results also highlight the potential use of butyrate as adjuvant in UC treatment and show that butyrate solution is an effective oral supplement.

References

[1] Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev 2001;81:1031–64.
[2] Ramos MG, Bambirra EA, Cara DC, Vieira EC, Alvarez-Leite JI. Oral administration of short-chain fatty acids reduces the intestinal mucositis caused by treatment with Ara-C in mice fed commercial or elemental diets. Nutr Cancer 1997;28: 212–7.
[3] Kanauchi O, Andoh A, Iwanaga T, Fujiyama Y, Mitsuyama K, Toyonaga A, et al. Germinated barley foodstuffs attenuate colonic mucosal damage and mucosal nuclear factor kappa B activity in a spontaneous colitis model. J Gastroenterol Hepatol 1999;14:1173–9.
[4] Patz J, Jacobsohn WZ, Gottschalk-Sabag S, Zeides S, Braverman DZ. Treatment of refractory distal ulcerative colitis with short chain fatty acid enemas. Am J Gastroenterol 1996;91:731–4.
[5] Scheppach W. Treatment of distal ulcerative colitis with short-chain fatty acid enemas. A placebo-controlled trial. German–Austrian SCFA Study Group. Dig Dis Sci 1996;41:2254–9.
[6] Scheppach W, Sommer H, Kirchner T, Paganelli GM, Bartram P, Christl S, et al. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 1992;103:51–6.
[7] Kanauchi O, Iwanaga T, Mitsuyama K, Saiki T, Tsuruta O, Noguchi K, et al. Butyrate from bacterial fermentation of germinated barley foodstuff preserves intestinal barrier function in experimental colitis in the rat model. J Gastroenterol Hepatol 1999;14:880–8.
[8] Videla S, Vilaseca J, Antolin M, Garcia-Lafuente A, Guarner F, Crespo E, et al. Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. Am J Gastroenterol 2001;96:1486–93.
[9] Roda A, Simoni P, Magliulo M, Nanni P, Baraldini M, Roda G, et al. A new oral formulation for the release of sodium butyrate in the ileo-cecal region and colon. World J Gastroenterol 2007;13:1079–84.
[10] Di Sabatino A, Morera R, Ciccocioppo R, Cazzola P, Gotti S, Tinozzi FP, et al. Oral butyrate for mildly to moderately active Crohn's disease. Aliment Pharmacol Ther 2005;22:789–94.
[11] Vernia P, Monteleone G, Grandinetti G, Villotti G, Di Giulio E, Frieri G, et al. Combined oral sodium butyrate and mesalazine treatment compared to oral mesalazine alone in ulcerative colitis randomized. Double-blind, placebo-controlled pilot study. Dig Dis Sci 2000;45:976–81.
[12] Smirick-Tjardes MR, Grieshop CM, Flickinger EA, Bauer LL, Fahey Jr GC. Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentive characteristics of growing pigs. J Anim Sci 2003;81:2535–45.
[13] Reeves PG, Nielsen FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.
[14] Lefrancois L, LeCorre R, Mayo J, Bluestone JA, Goodman T. Extrathymic selection of TCR gamma delta+T cells by class II major histocompatibility complex molecules. Cell 1990;63:333–40.
[15] Werner U, Szelenyi I. Measurement of MPO activity as model for detection of granulocyte infiltration in different tissues. Agents Actions 1992;38 [Spec No:C101–3].
[16] Van Nuenen MH, De Ligt RA, et al. The influence of microbial metabolites on human intestinal epithelial cells and macrophages in vitro. FEMS Immunol Med Microbiol 2005;45:183–9.
[17] Venkatraman A, Ramakrishna BS, Shaji RV, Kumar NS, Pulimood A, Patra S. Amelioration of dextran sulfate colitis by butyrate: role of heat shock protein 70 and NF-kappaB. Am J Physiol Gastrointest Liver Physiol 2003;285:G177–84.
[18] Wirtz S, Neurath MF, et al. Mouse models of inflammatory bowel disease. Adv Drug Deliv Rev 2007;59:1073–83.
[19] Elson CO, Cong Y, McCracken VJ, Dimmitt RA, Lorenz RG, Weaver CT. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunol Rev 2005;206: 260–76.
[20] Aguilar-Nascimento JE, Salomao AB, Nochi Jr RJ, Nascimento M, Neves Jde S. Intraluminal injection of short chain fatty acids diminishes intestinal mucosa injury in experimental ischemia-reperfusion. Acta Cir Bras 2006;21:21–5.
[21] Wedemeyer J, Vosskuhl K. Role of gastrointestinal eosinophils in inflammatory bowel disease and intestinal tumours. Best Pract Res Clin Gastroenterol 2008;22: 537–49.
[22] Coppi LC, Thomazzi SM, de Ayrizono ML, Coy CS, Fagundes WJ, Goes JR, et al. Comparative study of eosinophil chemotaxis, adhesion, and degranulation in vitro in ulcerative colitis and Crohn's disease. Inflamm Bowel Dis 2007;13:211–8.
[23] Lampinen M, Backman M, Winqvist O, Rorsman F, Rönnblom A, Sangfelt P, et al. Different regulation of eosinophil activity in Crohn's disease compared with ulcerative colitis. J Leukoc Biol 2008;84:1392–9.
[24] Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID). J Allergy Clin Immunol 2004;113:11–28.
[25] Rothenberg ME, Mishra A, et al. Gastrointestinal eosinophils. Immunol Reviews 2001;179:139–55.
[26] Wolever TM, Fernandes J, et al. Serum acetate:propionate ratio is related to serum cholesterol in men but not women. J Nutr 1996;126:2790–7.
[27] Kaneko M, Ishihara K, Takahashi A, Hong J, Hirasawa N, Zee O, et al. Mechanism for the differentiation of EoL-1 cells into eosinophils by histone deacetylase inhibitors. Int Arch Allergy Immunol 2007;143:28–32.
[28] Bailon E, Cueto-Sola M, Utrilla P, Rodrıguez-Cabezas ME, Garrido-Mesa N, Zarzuelo A, et al. Butyrate in vitro immune modulatory effects might be mediated through a proliferation-related induction of apoptosis. Immunology 2010.
[29] Cavaglieri CR, Nishiyama A, Fernandes LC, Curi R, Miles EA, Calder PC. Differential effects of short-chain fatty acids on proliferation and production of pro- and anti-inflammatory cytokines by cultured lymphocytes. Life Sci 2003;73:1683–90.
[30] Brandtzaeg P, Carlsen HS, Halstensen TS. The B-cell system in inflammatory bowel disease. Adv Exp Med Biol 2006;579:149–67.
[31] Ahrens R, Waddell A, Seidu L, Blanchard C, Carey R, Forbes E, et al. Intestinal macrophage/epithelial cell-derived CCL11/eotaxin-1 mediates eosinophil recruitment and function in pediatric ulcerative colitis. J Immunol 2008;181:7390–9.
[32] Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest 2007;117:514–21.
[33] Faria AM, Weiner HL. Oral tolerance. Immunol Rev 2005;206:232–59.
[34] Koruda MJ, Rolandelli RH, Bliss DZ, Hastings J, Rombeau JL, Settle RG. Parenteral nutrition supplemented with short-chain fatty acids: effect on the small-bowel mucosa in normal rats. Am J Clin Nutr 1990;51:685–9.
[35] Koruda MJ, Rolandelli RH, Settle RG, Zimmaro DM, Rombeau JL. Effect of parenteral nutrition supplemented with short-chain fatty acids on adaptation to massive small bowel resection. Gastroenterology 1988;95:715–20.
[36] Tian J, Hao L, Chandra P, Jones DP, Willams IR, Gewirtz AT, et al. Dietary glutamine and oral antibiotics each improve indexes of gut barrier function in rat short bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2009;296: G348–55.