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Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils☆

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Abstract

Short chain fatty acids (SCFAs) are fermentation products of anaerobic bacteria. More than just being an important energy source for intestinal epithelial cells, these compounds are modulators of leukocyte function and potential targets for the development of new drugs. The aim of this study was to evaluate the effects of SCFAs (acetate, propionate and butyrate) on production of nitric oxide (NO) and proinflammatory cytokines [tumor necrosis factor α (TNF-α) and cytokine-induced neutrophil chemoattractant-2 (CINC-2αβ)] by rat neutrophils. The involvement of nuclear factor κB (NF-κB) and histone deacetylase (HDAC) was examined. The effect of butyrate was also investigated in vivo after oral administration of tributyrin (a pro-drug of butyrate). Propionate and butyrate diminished TNF-α, CINC-2αβ and NO production by LPS-stimulated neutrophils. We also observed that these fatty acids inhibit HDAC activity and NF-κB activation, which might be involved in the attenuation of the LPS response. Products of cyclooxygenase and 5-lipoxygenase are not involved in the effects of SCFAs as indicated by the results obtained with the inhibitors of these enzymes. The recruitment of neutrophils to the peritonium after intraperitoneal administration of a glycogen solution (1%) and the ex vivo production of cytokines and NO by neutrophils were attenuated in rats that previously received tributyrin. These results argue that this triglyceride may be effective in the treatment of inflammatory conditions.

Keywords: Short chain fatty acids; Butyrate; Inhibitors of HDAC; Neutrophils and cytokines

1. Introduction

Short chain fatty acids (SCFAs) are produced in human gut by anaerobic fermentation of undigested carbohydrates and fibre polysaccharides. These fatty acids (mainly, acetate, propionate and butyrate) are found in intestine at high concentrations (from 70 to 140 mmol/L) [1,2], where they play a role as fuel for intestinal epithelial cells and as modulators of colonic blood flow [3], gastrointestinal (GI) motility [4] and fluid and electrolyte absorption [5].

SCFAs also have been implicated in pathological diseases that affect GI tract such as colon cancer and inflammatory bowel diseases (IBD) [6]. Modification in the concentrations of SCFAs in the colonic lumen has been reported in patients with IBD [6]. Studies on mice and humans showed that increased dietary fibre ingestion, which induces bacteria to produce more SCFAs, attenuates the colonic injury and intestinal inflammation [7,8]. Rectal enemas containing SCFAs mixtures, or just butyrate, has been shown (in most of the studies) to improve clinical and inflammatory parameters in the treatment of patients with ulcerative colitis [9]. A potential anti-inflammatory effect of SCFAs in other inflammatory conditions such as sepsis and rheumatoid arthritis has also been suggested [10,11].

Leukocytes and endothelial cells play a key role in the inflammatory process and their functions are modulated by SCFAs [11–13]. Most of these studies has focused on butyrate and reported inhibition of the production and release of inflammatory mediators such as cytokines, nitric oxide (NO) and lipid mediators by this fatty acid.

Neutrophils are the first effector cells recruited to the inflammatory site, where they internalize, kill and digest bacteria and fungi. Compared to lymphocytes and macrophages, neutrophils produce fewer molecules of cytokine per cell; nevertheless, they often outcome the number of mononuclear cells at inflammatory sites thus, neutrophils are important source of cytokines such as tumor necrosis factor α (TNF-α), interleukin (IL)-1β and IL-8 in the inflammatory response [14]. Additionally, neutrophils express inducible nitric oxide synthase (iNOS or NOS II) and cyclooxygenase (COX), enzymes responsible for generation of NO and eicosanoids, respectively. These soluble mediators are released in the inflammatory site

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and coordinate various steps of the inflammatory process such as recruitment and activation of leukocytes.

SCFAs affect different aspects of neutrophils biology such as intracellular pH [15] and calcium concentrations [16], production of reactive oxygen species, phagocytosis [17] and chemotaxis [16]. In spite of a number of studies on macrophages and endothelial cells, there is a lack of information about the effect of SCFAs on production of proinflammatory mediators by neutrophils. The only study that focused on this aspect reported reduction of TNF-α production by lipopolysaccharide (LPS)-stimulated neutrophils in the presence of SCFAs [18]. However, the mechanisms involved in the effects of the fatty acids such as activation of transcription factor and analyses of mRNA expression were not investigated in neutrophils. The aim of this study was to evaluate the effect of acetate, propionate and butyrate on production of NO and cytokines [TNF-α and cytokine-induced neutrophil chemoattractant-2 (CINC-2αβ)] by rat neutrophils. The involvement of nuclear factor κB (NF-κB) and histone deacetylase was also evaluated. The effect of butyrate was also investigated in vivo by oral administration of tributyrin (a pro-drug of butyrate).

2. Methods and materials

2.1. Reagents

Bovine fetal serum, HEPES, penicillin, streptomycin, sodium bicarbonate, fatty acids, tributyrin, LPS (*Escherichia coli* strain 0111:B4), oyster glycogen and RPMI-1640 medium supplemented with L-glutamine were supplied by Sigma Chemical (St. Louis, MO, USA). Indometacin, etoricoxib and zileuton were kindly provided by Dra. Catarina de F. Teixeira (Butantan Institute). Stock solutions of the fatty acids were prepared in phosphate-buffered saline (PBS) and pH was adjusted to 7.4 with NaOH solution (1 N).

2.2. Animals

Male Wistar rats weighing 180±20 g were obtained from the Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, Brazil. The rats were maintained at 23°C under a light: dark cycle of 12:12 h. Food and water were given ad libitum. The Animal Care Committee of the Institute of Biomedical Sciences approved the experimental procedure of this study.

2.3. Neutrophil preparation

Rat neutrophils were obtained by intraperitoneal lavage with 30 ml PBS, 4 h after intraperitoneal injection of 10 ml 1% (w/v) sterile glycogen solution (Sigma type II, from oyster) in PBS. The cell suspension was centrifuged at 4°C (500×*g* for 10 min). The number of viable cells (>95% neutrophils) was determined in a Neubauer chamber under an optical microscope by Trypan blue exclusion.

2.4. Culture conditions

Cells were mantained in RPMI-1640 culture medium containing 10% fetal bovine serum (FBS), glutamine (2 mmol/L), HEPES (20 mmol/L), streptomycin (100 μg/ml), penicillin (100 international U/ml) and sodium bicarbonate (24 mmol/L). In order to remove macrophage contamination, cells were pre-incubated in culture flask for 1 h, and then nonadherent cells were collected and used for the assay. Cells (2.5×10^6 cells/ml) were incubated in 24-well polystyrene culture plates, at 37°C and 5% CO_2, with acetate (10 and 25 mmol/L), propionate (0.4, 4, 8 and 12 mmol/L) or butyrate (0.4, 0.8, 1.6 and 3.2 mmol/L), with or without 5 μg/ml LPS. Cell supernatant was collected at the end of the incubation period (4 or 18 h) and stored at −80°C until the measurement of cytokines and NO.

For the experiments using COX and 5-lipoxygenase (LOX) inhibitors, cells were pretreated with COX inhibitors, 10 μmol/L indometacin (nonselective COX inhibitor) or 2.5 μmol/L etoricoxib (COX-2 selective inhibitor), LOX inhibitor and 2.5 μmol/L zileuton for 1 h before addition of the SCFAs and LPS. Concentrations of LOX and COX inhibitors used in this experiment were chosen taking into account previous studies [19,20].

2.5. NO production

For NO determination, nitrite content was measured using method described by Ding et al. [21]. Briefly, supernatants (100 μl) were incubated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylene diamine dihydrochloride, 2.5% H_3PO_4) at room temperature, for 10 min, and absorbance read at 550 nm. Sodium nitrite was used as standard for calibration curve. The cell-free medium contained 0.2–0.3 nmol NO_2^- per well; this value was determined in each experiment and subtracted from that obtained with cells.

2.6. Cytokine quantification

Concentrations of TNF-α and CINC-2αβ were determined in cell culture supernatants using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA).

2.7. Determination of TNF-α, CINC-2 and iNOS mRNA levels

The effect of SCFAs on mRNA levels of iNOS and cytokines (TNF-α and CINC-2) was evaluated in neutrophils incubated for 1 or 4 h with acetate (25 mmol/L), propionate (12 mmol/L) or butyrate (1.6 mmol/L) and LPS (5 μg/ml). Total RNA was obtained from 1.5×10^7 neutrophils using Trizol reagent (Invitrogen Life Technologies, Rockville, MD, USA), as previously described by Chomczynski and Sacchi [22].

Total RNA (3 μg) was reverse transcribed to cDNA using reverse transcriptase *Revertaid* M-MuLV. Expression of iNOS and cytokines was evaluated by real-time polymerase chain reaction [23] using a Rotor Gene 3000 equipment (Corbett Research, Mortlake, Australia) and SYBR Green as fluorescent dye. The primers sequence is shown in Table 1. Quantification of gene expression was carried out using the method described by Liu and Saint [24] with β2 microglobulin gene as inner control.

2.8. Measurement of histone deacetylase (HDAC) activity

HDAC activity was measured by using a fluorescence assay kit (Cayman Chemical, USA). Nuclear extracts of neutrophils (5 μg protein) were incubated with acetylated fluorogenic substrate (25–200 μmol/L) in 160 μl assay buffer in the presence or absence of various concentrations of acetate (10, 25 and 100 mmol/L), propionate (0.1, 1, 10, 25 and 100 mmol/L) and butyrate (0.1, 1, 10, 25 and 100 mmol/L). All compounds were dissolved in water and neutralized before dilution with HDAC assay buffer (25 mmol/L Tris–HCl, pH 8.0, 137 mmol/L, NaCl, 2.7 mmol/L KCl and 1 mmol/L $MgCl_2$). The deacetylation reaction was carried out at 37°C for 30 min and stopped by addition of 40 μl HDAC developer. Fifteen minutes after stopping the reaction, fluorescence was measured using a Fluorocount reader (Packard BioScience, Meriden, CT, USA) at 360-nm excitation and 465-nm emission. The measurement of HDAC activity in the absence of fatty acids or trichostatin A (TSA) was considered as the maximal activity (100%). Results were then expressed as percentage of maximal activity.

2.9. Electrophoretic mobility shift assay

NF-κB activation was evaluated after treatment of the cells for 30 min with acetate, propionate or butyrate at 25, 12 and 1.6 mmol/L, respectively, in the presence of LPS (5 μg/ml). Nuclear extract from neutrophils was obtained as previously described [25]. Double-stranded oligonucleotides containing the NF-κB consensus binding site (5′-AGTTGAG*GGGACTTTCC*CAGGC-3′) [26] were end-labeled using T4 polynucleotide kinase and [γ-^{32}P]ATP (Amersham Biosciences, NJ, USA). Binding reactions of the probes were performed with 5 μg proteins from nuclear extract, at room temperature, for 20 min, in 12 μl binding buffer consisting of 20 mmol/L HEPES, pH 7.6, 50 mmol/L KCl, 10% glycerol, 0.2 mmol/L EDTA, 1 mmol/L DTT and 2 μg polydeoxyinosinic–deoxycytidylic acid. Competitive binding assays were conducted under same conditions with the addition of 2 pmol (100-fold molar excess) unlabeled competitor oligonucleotide. The DNA–protein complexes were electrophoresed on 4% nondenaturing polyacrylamide gels, at 4°C, in 45 mmol/L Tris, 45 mmol/L borate and 1 mmol/L EDTA buffer. The gels were dried and subjected to autoradiography. The blots were analysed by scanner densitometry (Image Master 1D, Amersham Biosciences), and results were expressed as percentage of control condition (PBS plus LPS).

Table 1
The annealing temperature and sequences of the primers used are given

Gene	Primer sense	Primer antisense	Annealing temperature
β2 microglobulin	CTCAGTTCCACCCACCTCAG	GCAAGCATATACATCGGTCTCG	56°C
iNOS	GGATATCTTCGGTGCGGTCTT	GCTGTAACTCTTCTGGGTGTCAGA	58°C
CINC-2	TGTACTGGTCCTGCTCCTCCTG	GGGCTTCAGGGTTGAGACAAAC	60°C
TNF-α	GCCTCTTCTCATTCCTGCTCGTGG	TTCTCCTCCTTGTTGGGACCGATC	60°C

2.10. Treatment of animals with tributyrin

Rats were orally treated with a single dose of 3.6 g/kg body weight of tributyrin. Under this dose of tributyrin, plasma butyrate concentration reaches up to 0.4 mmol/L [27]. After 1 h of tributyrin administration, animals were intraperitoneally injected with oyster glycogen solution 1% (w/v). Four hours later, neutrophils were collected, counted in a Neubauer chamber and assayed for production of cytokines and NO, as described.

2.11. Statistical analysis

Comparisons were performed using one-way analysis of variance and Dunnett's multiple comparison post test. The significance was set at $P<.05$. Results were obtained from at least three separate experiments and expressed as means±S.E.M.

3. Results

Production of proinflammatory cytokines (TNF-α and CINC-2αβ) and NO was evaluated in LPS-stimulated neutrophils incubated during 4 (TNF-α) and 18 h (NO and CINC-2αβ) in the presence of various concentrations of acetate, propionate or butyrate. All experiments were carried out using nontoxic concentrations of SCFAs as determined in previous experiments by flow cytometry using propidium iodine to measure membrane integrity and DNA fragmentation (data not shown). The concentrations of SCFAs used are higher than those found in blood (2–200 μmol/L) but are in the range of concentrations found in the GI tract (from 70 to 140 mmol/L) and in sites of infection by anaerobic bacteria, where SCFAs may regulate the inflammation in conditions such as IBD and periodontal disease [1,2,28,29].

LPS significantly increased the production of the cytokines and NO by neutrophils (Fig. 1). Acetate had no effect on production of TNF-α, CINC-2αβ or NO by neutrophils (Fig. 1A, D and G). However, propionate (4 mmol/L and above) and butyrate (0.4 mmol/L and above) reduced the production of TNF-α and CINC-2αβ (Fig. 1B, C, E and F). Propionate (12 mmol/L) and butyrate (1.6 mmol/L and above) also inhibited NO production by LPS-stimulated neutrophils (Fig. 1H and I). The production of cytokines and NO by neutrophils not stimulated with LPS was not affected by the fatty acids (data not shown).

In order to investigate the mechanism involved in the inhibition of LPS-stimulated production of cytokines and NO by propionate and butyrate, the mRNA levels of TNF-α, CINC-2 and iNOS were determined. In this experiment, LPS-stimulated neutrophils were treated with SCFAs for 1 (TNF-α) or 4 h (iNOS and CINC-2). The fatty acids were used in this and in other experiments in the lowest concentrations that inhibited production of cytokines and NO (12 mmol/L of propionate and 1.6 mmol/L of butyrate). Acetate, which had no effect on TNF-α, CINC-2αβ or NO production, was added at the highest non-toxic concentration (25 mmol/L). Propionate and butyrate reduced TNF-α and CINC-2 mRNA levels (Fig. 2A and B). LPS-stimulated expression of iNOS mRNA was inhibited by butyrate (Fig. 2C). Neither propionate nor acetate modified the iNOS mRNA expression (Fig. 2C).

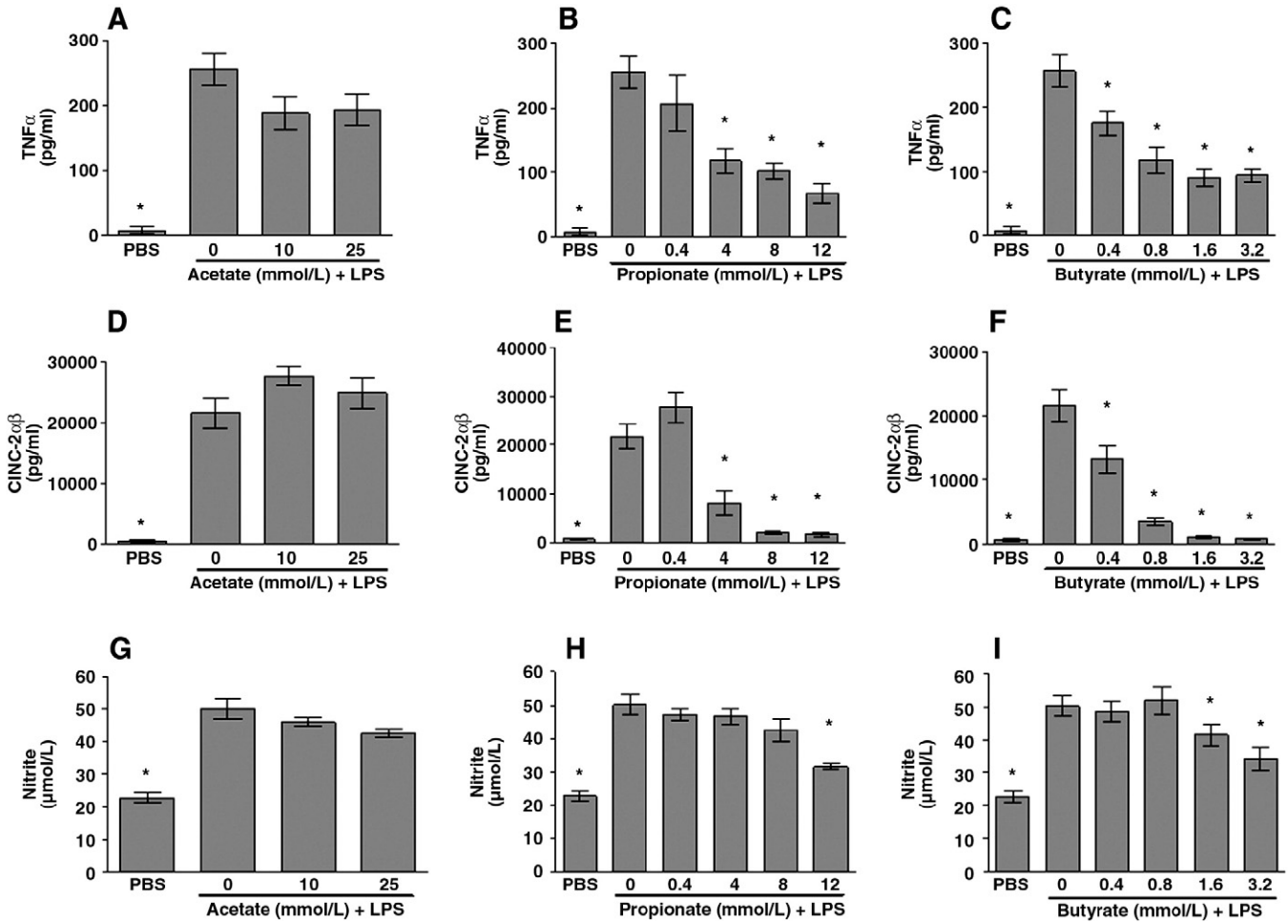

Fig. 1. Effect of the SCFAs on production of cytokines and NO by neutrophils. TNF-α, CINC-2αβ and nitrite were measured in the supernantant of neutrophils incubated during 4 (TNF-α) and 18 h (CINC-2αβ and nitrite) with various concentrations of the SCFAs (mmol/L) and LPS (5 μg/ml). Results are presented as means±S.E.M. of four experiments carried out in duplicate. *$P<.05$ as compared to cells treated with LPS.

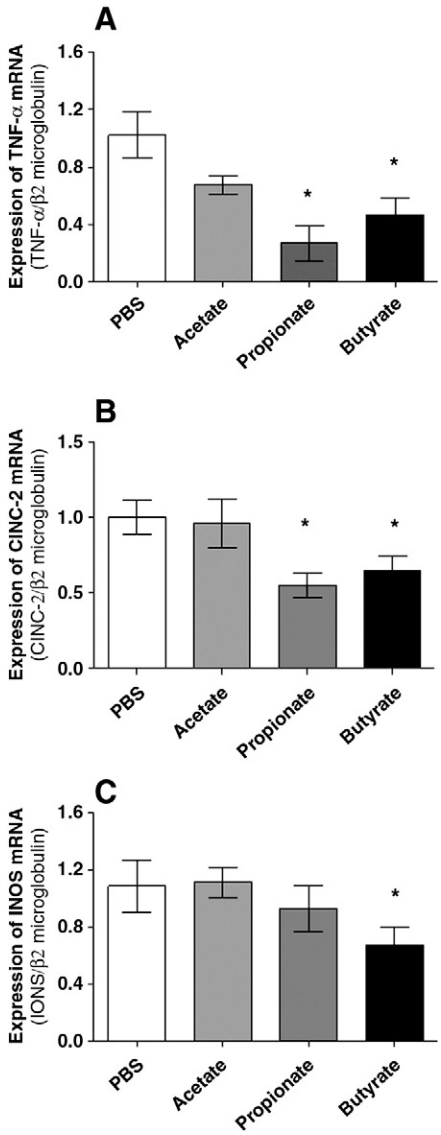

Fig. 2. Effect of the SCFAs on mRNA levels of TNF-α, CINC-2αβ and iNOS. Expression of cytokines and iNOS mRNA was carried out in LPS-stimulated (5 μg/ml) neutrophils incubated for 1 (TNF-α) and 4 h (CINC-2αβ and iNOS) with acetate (25 mmol/L), propionate (12 mmol/L) or butyrate (1.6 mmol/L). Results are presented as means ±SEM of three experiments carried out in duplicate. *P<.05 as compared to cells treated with PBS.

The effect of various concentrations of acetate, propionate and butyrate on HDAC activity was tested. SCFAs inhibited HDAC activity (Fig. 3). Butyrate was the most potent inhibitor with an IC_{50} of 2.8 mmol/L. Propionate presented an IC_{50} of 8.7 mmol/L and the less potent was acetate (IC_{50}=66 mmol/L). We also investigated the effect of TSA, a potent inhibitor of HDAC activity [30], on production of cytokines and NO by neutrophils. TSA, at 25 nmol/L, significantly reduced the production of TNF-α, CINC-2αβ and NO by LPS-stimulated neutrophils (Fig. 4), indicating that HDAC inhibition may be the mechanism by which propionate and butyrate modulate the expression of inflammatory mediators in neutrophils.

The effect of SCFAs on activation of NF-κB, a pivotal transcription factor involved in expression of proinflammatory genes, was also investigated. The activation of this transcription factor was evaluated after stimulation of neutrophils with LPS for 30 min in the presence of the SCFAs. Consistent with other results, propionate and butyrate attenuated the activation of NF-κB by LPS, whereas acetate had no

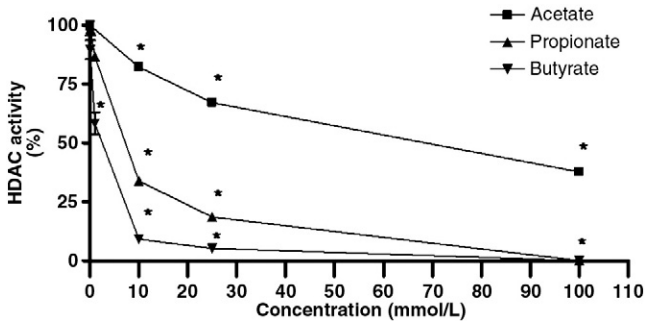

Fig. 3. Effect of SCFAs on HDAC activity. HDAC activity of neutrophil nuclear extracts was measured in the presence of various concentrations of SCFAs. Results are presented as means±S.E.M. of two experiments carried out in duplicate. *P<.05 as compared to cells treated with PBS (control).

effect (Fig. 5). SCFAs did not affect the activation of NF-κB in the absence of LPS (data not shown).

The possible participation of prostaglandins and leukotrienes in the inhibitory effect of propionate and butyrate was investigated. Neutrophils were pretreated with COX inhibitors, indometacin (non selective COX inhibitor) or etoricoxib (COX-2 selective inhibitor) or LOX inhibitor, zileuton, for 1 h. Cells were stimulated with LPS in the presence of the SCFAs and the production of cytokines and NO were measured. COX or LOX inhibition had no effect on propionate and butyrate attenuation of LPS-stimulated production of TNF-α (Fig. 6).

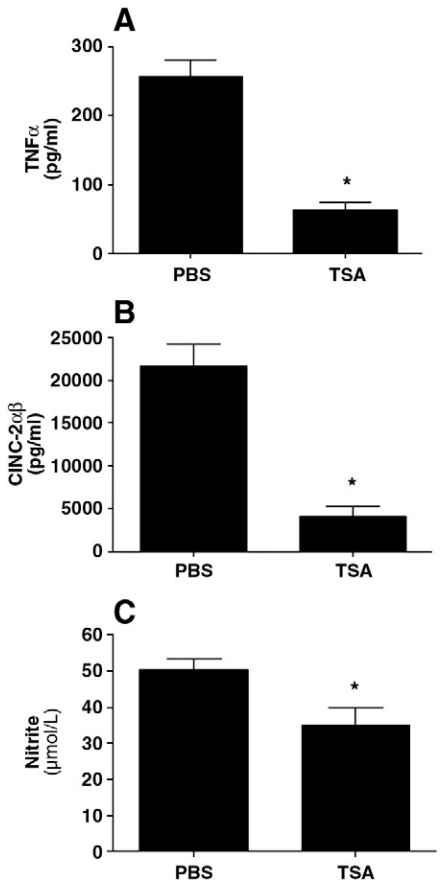

Fig. 4. Effect of TSA on production of TNF-α, CINC-2αβ and NO. Cytokines and nitrite were measured in the supernantant of LPS-stimulated (5 μg/ml) neutrophils incubated for 4 (TNF-α) and 18 h (CINC-2αβ and nitrite) with TSA (25 mmol/L). Results are presented as means±S.E.M. of four experiments carried out in duplicate. *P<.05 as compared to cells treated with PBS.

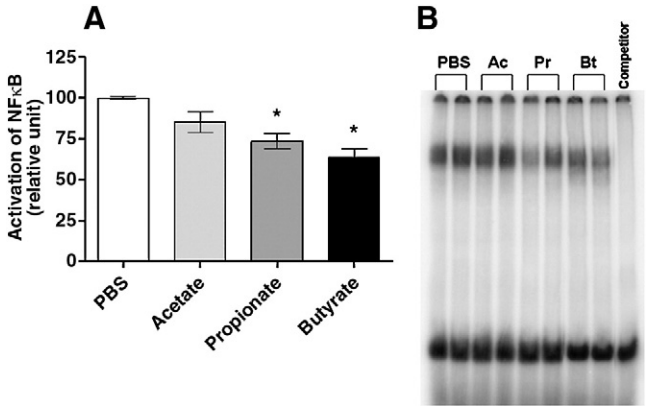

Fig. 5. Effect of the SCFAs on NF-κB activation. Nuclear extracts obtained from LPS-stimulated (5 μg/ml) neutrophils incubated with acetate (25 mmol/L), propionate (12 mmol/L) or butyrate (1.6 mmol/L) for 30 min were used for protein–DNA binding assays in the presence of the radio-labeled probe (30,000 cpm). (A) Results are expressed as relative values and compared to cells treated with LPS only. Results are presented as means±S.E.M. of three experiments carried out in duplicate. *P<.05 as compared to cells treated with PBS (control). (B) Representative figure of the experiments.

Similar results were observed for CINC-2αβ and NO production (data not shown).

Considering the fact that butyrate was the most potent inhibitor of the production of proinflammatory mediators by isolated neutrophils, the effect of this SCFA was also tested in vivo. Tributyrin, a prodrug of butyrate, which has more favorable pharmacokinetic properties and is better tolerated than butyrate [31] was orally given to rats. Administration of tributyrin reduced the recruitment of neutrophils to the peritonium (inflammatory site) in response to intraperitoneal injection of glycogen (Fig. 7A). Tributyrin treatment also inhibited ex vivo production of proinflammatory cytokines (TNF-α and CINC-2αβ) and NO by LPS-stimulated neutrophils (Fig. 7B–D).

4. Discussion

Evidence is presented herein that butyrate has anti-inflammatory effects; this SCFA inhibited both in vitro and ex-vivo production of proinflammatory cytokines (TNF-α and CINC-2αβ) and NO by LPS-stimulated neutrophils. Propionate also reduced the production of these mediators in vitro by neutrophils but was less potent than butyrate. Inhibition of HDAC activity and NF-κB activation may participate in the effects of propionate and butyrate in neutrophils.

SCFAs regulate neutrophil functions such as production of reactive oxygen species, phagocytosis [17] and chemotaxis [16,32]. Activation of G-protein coupled receptor 43, which couples to Gi/o and Gq proteins, leads to intracellular Ca^{2+} mobilization and is involved in the chemotatic effect of SCFAs on neutrophils [16,33]. SCFAs can also act on leukocytes through inhibition of HDAC activity [34]. This enzyme together with histone acetyltransferase (HAT) controls chromatin acetylation. A common view has emerged that histone acetylation, which reduces the affinity between histone proteins and DNA, facilitating the binding of transcription factors to DNA, enhances transcription, whereas histone deacetylation is associated with transcription repression [35]. In addition to histones, other proteins are regulated by acetylation such as STAT-3, glucocorticoid receptor and NF-κB [36–38]. Our hypothesis is that propionate and butyrate by inhibiting HDAC activity modulate the activation of transcription factors such as NF-κB and consequently the expression of genes involved in the inflammatory response. The correlation between

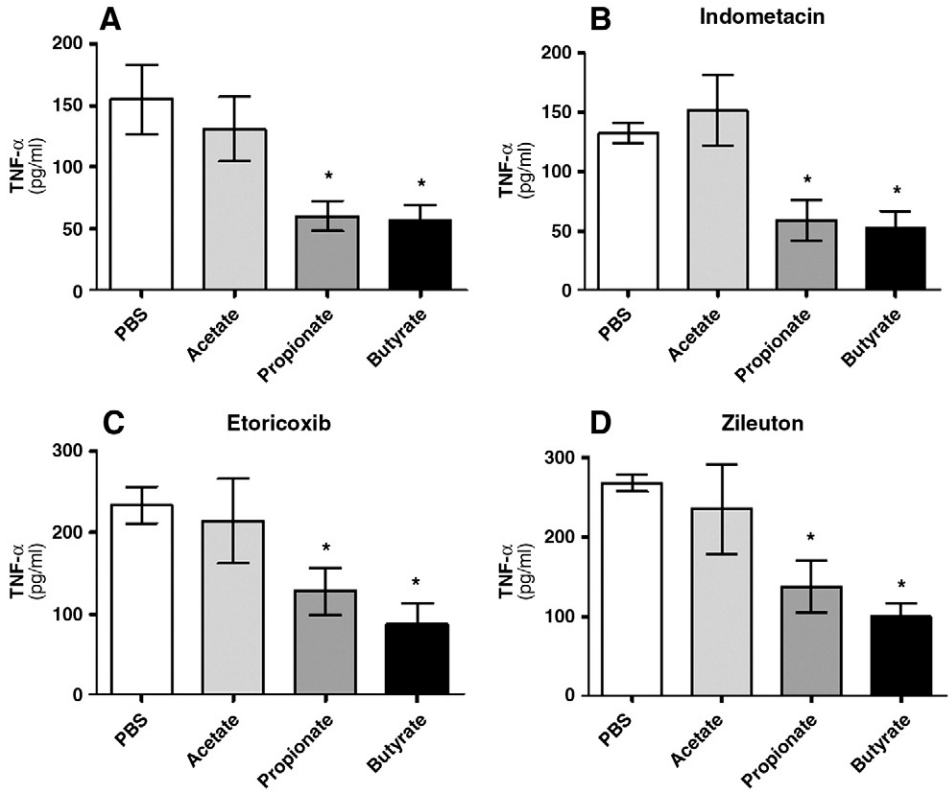

Fig. 6. Effect of SCFAs on production of TNF-α by neutrophils pretreated with COX (indometacin and etoricoxib) or LOX (zileuton) inhibitors. TNF-α was measured in the supernantant of LPS-stimulated (5 μg/ml) neutrophils previously incubated with indometacin (10 μmol/L), etoricoxib (2.5 μmol/L) or zileuton (2.5 μmol/L) for 1 h and then with acetate (25 mmol/L), propionate (12 mmol/L) or butyrate (1.6 mmol/L). Results are presented as means±S.E.M. of three experiments carried out in duplicate. *P<.05 as compared to cells treated with PBS.

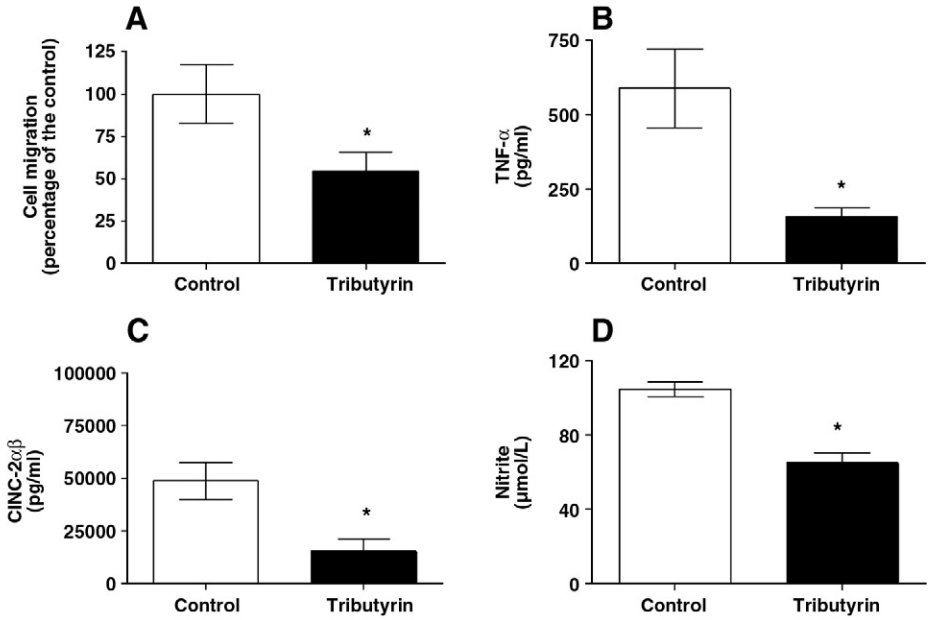

Fig. 7. Effect of tributyrin given by gavage on migration of neutrophils to inflammatory site and production of cytokines and NO by these cells. The number of cells that migrated to the inflammatory site was measured 4 h after injection of oyster glycogen solution. Production of cytokines and NO by LPS-stimulated (5 μg/ml) neutrophils was also measured 4 (TNF-α) or 18 hours (CINC-2αβ and nitrite) after incubation. Results are presented as means±SEM of five experiments. *$P<.05$ as compared with control.

HDAC activity and NF-κB activation has already been demonstrated, but this interaction is not completely understood. Both direct acetylation of the transcription factor and modulation of other signalling proteins involved in the NF-κB pathway have been shown to be involved [39,40].

Previous studies also reported that propionate and butyrate inhibit the production of proinflammatory cytokines and NO by macrophages, an effect associated to inhibition of NF-κB activation [12,41]. In neutrophils, Tedelind et al. [18] have shown that acetate, propionate and butyrate at 30 mmol/L decrease the production of TNF-α without affecting IL-8 release. In the present study, we did not observe any effect of acetate (10 and 25 mmol/L) on production of TNF-α by neutrophils. However, we did notice that propionate and butyrate reduce the production of CINC-2αβ, which, as human IL-8, is a potent neutrophil chemoattractant agent in rat [42].

Usami et al. [37] have found that SCFAs attenuate the production of TNF-α by human blood mononuclear cells. The authors demonstrated that butyrate and propionate inhibit TNF-α production via stimulation of prostaglandin E_2 production and down-regulation of LPS induced activation of NF-κB. Usami et al. [37] also reported that acetate reduces TNF-α production by monocytes. According to these authors this last effect is associated with LOX activation. These observations lead us to examine the participation of COX and LOX products in the effects of SCFAs on neutrophils. However, no interference of COX or LOX inhibitors in the effects of SCFAs on neutrophils was observed.

Evidence is presented herein that butyrate inhibits the inflammatory process; oral administration of tributyrin, a prodrug of butyrate, attenuated the inflammation caused by glycogen injection. The influx of neutrophils into the peritonium after glycogen injection has previously been shown to be dependent on expression of adhesion molecules (selectins and β2 integrins), cytokines and chemokines [43]. Butyrate reduced the migration of neutrophils possibly by inhibiting the production of cytokines and chemokines in the inflammatory site. Tributyrin treatment reduced the production of inflammatory mediators by macrophages (unpublished data) and neutrophils. As previously described by us and others [32,44,45], butyrate also regulates the expression of adhesion molecules and migration of neutrophils to tissues. However, this effect is unlikely to be involved in the attenuation of neutrophil recruitment since tributyrin treatment did not reduce the number of rolling or adhered leucocytes to endothelium as evaluated by intravital microscopy (unpublished data).

Butyrate presents beneficial effects on inflammatory conditions such as ulcerative colitis and sepsis [9,10]. These effects are attributed, at least in part, to attenuation of production of inflammatory mediators. This SCFA, however, has a very short half-life requiring large intravenous volume and continuous administration to reach effective concentrations in blood [27,46]. Oral administration of tributyrin, a triglyceride containing three butyrate moieties esterified to a glycerol molecule, maintains substantial butyrate plasma concentrations for a prolonged period of time [27]. Although clinical studies have been conducted in patients with cancer using tributyrin [47,48], at our knowledge, this is the first study to investigate the effect of this triglyceride on inflammation in vivo.

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