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Alimentary Tract

Effect of butyrate enemas on gene expression profiles and endoscopic/histopathological scores of diverted colorectal mucosa: A randomized trial

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ABSTRACT

Background: A temporary stoma is often created to protect a distal anastomosis in colorectal surgery. Short-chain fatty acids, mainly butyrate, are the major fuel source for the epithelium and their absence in the diverted tract may produce mucosal atrophy and inflammation.
Aims: To investigate whether the administration of sodium butyrate enemas (Naburen©, Promefarm, Italy) could prevent mucosal inflammation and atrophy and affect gene expression profiles after ileo/colostomy.
Methods: We performed a randomized, double-blind, placebo-controlled clinical trial, in patients with enterostomy performed for inflammatory bowel disease, colorectal cancer or diverticulitis. Twenty patients were randomly allocated to receive 30 ml of sodium butyrate 600 mmol/L (group A) or saline (group B), b.i.d. for 30 days.
Results: In group A endoscopic scores were significantly improved ($p < 0.01$) while mucosal atrophy was reduced or unchanged; in group B mucosal atrophy was increased in 42.8% of patients. Despite the high dose of butyrate used, no short-chain fatty acids were detectable by gas chromatography–mass spectrometry in colorectal biopsies. Group A patients showed up-regulation of genes associated with mucosal repair such as Wnt signalling, cytoskeleton regulation and bone morphogenetic protein-antagonists.
Conclusion: Butyrate enemas may prevent the atrophy of the diverted colon/rectum, thus improving the recovery of tissue integrity.

1. Introduction

Butyrate and other short-chain fatty acids (SCFAs) are an important energy source for the colonic epithelium and a chronic lack of luminal SCFAs may lead to a nutritional deficiency of the colonic epithelium, causing mucosal atrophy and deprivation colitis [1,2]. Diversion colitis may occur as a complication in a surgically diverted intestine [3] and is characterized by severely decreased luminal concentrations of SCFAs measured in the bypassed part of the rectosigmoid [4].

The administration of butyrate enemas in patients with inflammatory bowel disease (IBD) has produced contradictory results, perhaps related to the various experimental designs and patient compliance [5]. After 2 weeks of 100 mmol/L butyrate irrigation in 10 ulcerative colitis patients unresponsive or intolerant to standard therapy, stool frequency and histological inflammation decreased significantly [6]. In another study, 6/10 patients responded positively to butyrate enemas and 4 went into remission [7]; in a second study on 9 patients, endoscopic and histological improvement was observed in 7 patients after 2 weeks of therapy with 5-ASA and sodium butyrate [8]. A larger, 6-week, double-blind, placebo-controlled trial on 91 patients demonstrated an improvement in 33% of patients treated with SCFA enemas compared with 20% receiving placebo [9].

The use of SCFAs or butyrate enemas in patients with diversion colitis has been tried in few studies. Enemas containing SCFAs (60 mmol/L acetate; 30 mmol/L propionate; 40 mmol/L butyrate) administered twice a day for 14 days to 13 patients with excluded colon after various diseases did not ameliorate the endoscopic and histologic scores [10]. However, in a single blind cross-over trial,

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8 patients had SCFA irrigation of the closed rectal stump after resection of the sigmoid colon with an end colostomy (Hartmann's procedure), and increased proliferation activity was observed in all of them [11]. Similar results appeared in 4 patients with diversion colitis after SCFA irrigation [12].

The beneficial effect of SCFAs/butyrate enemas still requires confirmation and possibly mechanistic interpretation and information on topic butyrate alone is not available on diversion colitis. We here report the effects of relatively high concentrations (600 mmol/L) of butyrate in a small, double-blind study involving patients with endostomy and diverted colorectum. The primary aim of the trial was to assess the efficacy of butyrate in improving endoscopic and histological features of the patients. The secondary aim was to study the effect of butyrate on the global gene expression of the colorectal mucosa.

2. Patients and methods

2.1. Study design

The study design was a randomized, double-blind, placebo-controlled, parallel-group clinical trial (Study registration number PMF603-IS1/08). All patients admitted from December 2008 to November 2010 were recruited. All patients admitted to the Digestive Surgery Unit (Careggi University of Florence Hospital), at least 18 years old, operated at least 30 days previously for diverticular disease, cancer or IBD, with no concomitant medications, were considered eligible. Patients with surgical emergencies (occlusion, haemorrhage, peritonitis) were excluded. Eligible adult patients with enterostomy due to IBDs, colorectal cancer or diverticulitis were randomly assigned to either the intervention or control groups. Patients in the intervention group (group A) were administered an enema (Naburen©, Promefarm, Italy) containing sodium butyrate (2 g/30 ml; 600 mmol/L), twice daily for 30 days, while the saline group (group B) received the same volume of saline, containing 0.01 g/30 ml (3 mM) of sodium butyrate in order to confer the characteristic odour of butyrate to the solution, and thereby maintain the study blindness.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the Ethical Review Committee of the Hospital of Careggi, Florence. Written informed consent was obtained from all eligible participants.

The primary outcomes of the trial were to assess the safety of butyrate at high concentrations and its efficacy in improving endoscopic and histological features of the patients by increasing butyrate concentrations in the bypassed rectosigmoid.

The secondary endpoint was to study the effect of butyrate on the whole gene expression of the colorectal mucosa.

2.2. Endoscopic and histological grading

Recto-sigmoidoscopic (Olympus Tokyo, Japan) examination was performed in all patients involved in the study, on day 1 (at baseline), and on day 30 (at end of treatment), to monitor their rectal mucosal status. The endoscopic grade was defined as follows: 0 (normal-appearing mucosa and good distensibility), 1 (oedema and hyperaemia of the mucosa and good distensibility), 2 (loss of normal vascular pattern and erosion, reduced distensibility), 3 (mucosal ulcerations, stenosis, and loss of distensibility).

Rectal or colonic biopsy samples, 5–10 cm from the dentate line, were collected on days 1 and 30. One biopsy was fixed in buffered formalin and processed for histopathological examination, the second was stored in RNAlater (Qiagen, Milan, Italy) for RNA extraction and the third was frozen for SCFA determination.

Paraffin-embedded rectal biopsy sections stained with haematoxylin and eosin were examined by a pathologist (FZ). Histological grading was defined as follows: 0 (absence of atrophy or polymorphonuclear neutrophil (PMN) infiltration, 1 (mild atrophy or PMN infiltration <50% of 5 crypts), 1.5 (mild atrophy and infiltration of PMN <50% of 5 crypts), 2 (severe atrophy or PMN infiltration <50% of 5 crypts), 2.5 (severe atrophy and infiltration of PMN <50% of 5 crypts), 3 (mucosal erosions or ulcers of the intestinal mucosa), 3.5 (erosions and ulcers of the intestinal mucosa).

2.3. Biochemical analyses

For safety evaluation, the biomarker levels for kidney (urea and creatinine) or liver function (alanine transaminase and γ-glutamyl transferase) were assessed on days 1 and 30.

2.4. SCFA determination

SCFA determination was performed using a gas chromatograph (GS, Star 3400 Cx, Varian) coupled to a mass spectrometer (MS) with ion trap (Saturn 2000, Varian) on biopsies taken on days 1 and 30. The biopsies were weighed and inserted in a vial containing 500 μl of 10% (v/v) perchloric acid and 0.5 n/μl of deuterated internal standards (d^4 acetic, d^3 propionic and d^7 butyric). The samples were homogenized using an Ultraturrax homogenizer and centrifuged at 13,000 rpm at 4 °C. The supernatant (400 μl) was divided into 4 aliquots of 100 μl: two aliquots were immediately analyzed by GC–MS and the remaining were stored at −80 °C for subsequent analysis. The analyses were performed under the following conditions: acetonitrile carrier gas: silica fibre filters coated with Carboxen/polydimethyl siloxane polymer (CAR/PDMS, plain black, 75 μm thick, maximum temperature 320 °C, conditioning temperature 300 °C) as stationary phase. The fibre was periodically subjected to cleaning cycles before and after analysis. Truck temperature was 70 °C, injector temperature 290 °C, oven temperature program: starting at 60 °C (3 min), reaching 123 °C in 3 min, increasing to 159 °C (6 °C/min), and finally to 200 °C (20 °C/min). The capillary column was fused silica coated with PEG (stationary phase, polar), 30 m long HP-INNOWax (J & W GC-columns, Agilent), with internal diameter of 0.25 mm and inner film of 0.25 μm. The temperature of the transfer line was 256 °C, the analyzer ion trap temperature was 185 °C and the ionization mode a chemical ionization (CI) which provides less fragmentation of the molecules but a higher analyte signal and greater probability of seeing the molecular peak. The program used to perform all the experiments was the Varian MS Workstation, version 6.9.1. A calibration curve was prepared by adding the mixture of internal standards with different amounts of each acid; SCFA concentration in biopsies was expressed in ng per milligram/wet weight of tissue.

2.5. Transcriptomic analysis

Thirteen cases were randomly selected for transcriptomic analysis (7 from group A and 6 from group B). Total RNA was extracted using the RNeasy Mini kit plus (Qiagen, Milan, Italy). The gene expression profile analyses were performed using the Agilent 4 × 44K Whole Human Genome Microarray (Agilent Technologies, Palo Alto, CA, USA). The hybridization steps were carried out according to the Agilent protocol (Two-Color Microarray-Based Gene Expression Analysis version 5.7) using a two-color microarray protocol in which biopsies harvested at baseline (day 1) were contrasted, within each patient, with biopsies harvested after treatment (day 30). Images were scanned using a Genepix 4000B microarray scanner, at 5-μm resolution (Axon Instruments, Foster City, CA, USA). Image analysis and initial quality control were performed using Agilent Feature Extraction Software v9.5.

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Initial statistical analysis was performed using unpaired *t*-test considering Benjamini–Hochberg corrected *p*-value of 0.05. Functional analysis was performed using GO-elite version 1.2 beta (http://www.genmapp. org/go_elite). BRB-Array Tools Version 3.8.0 (http://linus.nci.nih.gov/BRB-ArrayTools.html) was used to perform Statistical Analysis of Microarray (SAM) and Gene Set Expression analysis (GSEA).

The microarray data sets supporting the results of this article are available in the MIAME public database ArrayExpress repository http://www.ebi.ac.uk/arrayexpress) Experiment name: BUTYRATE ENEMAS ON PATIENTS WITH ENTEROSTOMY ArrayExpress accession: E-MTAB-2436.

2.6. RT-PCR

100 ng of total RNA from each sample were reverse-transcribed using 100 units of SuperScript™ II Reverse Transcriptase (Life Technologies, Milan, Italy) and 1× random examers (Roche Diagnostics, Monza, Italy). Each gene was co-amplified with *GAPDH* as internal standard. PCRs were carried out using 2 μl of cDNA in a 25 μl total volume containing 1× PCR buffer, 1× Coral Dye, 0.5 mM dNTPs, 8 ng/μl of primer, 0.1 ng/μl of *GAPDH* primers and 1.25 units of Taq polymerase (Qiagen, Milan, Italy). Primer sequences are reported in Supplementary Table S1.

The PCR conditions were 95 °C for 7 min and 35 cycles at 95 °C for 30 s, 60 °C for 30 s and 72 °C for 55 s. PCR products were separated on a 1.8% agarose gel and visualized by ethidium bromide. Gel images were captured by a digital photocamera and the intensity of the bands analyzed with the Quantity-One software (Bio-Rad, Segrate, Milan, Italy).

2.7. Statistics

On the basis of previous studies, an improvement of 50% in the endoscopic score was assumed. With a power of 90% and a significance level of 0.05, the difference between groups A and B could have been statistically significant with 10 patients in each group. Calculations were performed with GraphPad Prism 5.0 program (GraphPad, San Diego, CA). Differences in the endoscopic and histopathological scores and on RT-PCR data were analyzed using the Wilcoxon matched pairs *t*-test and considered significant if $p \leq 0.05$.

3. Results

Table 1 reports the characteristics of the recruited patients who completed the trial.

Overall 20 patients were enrolled: 10 patients in group A (mean age 60.0 ± 4.8 years, 80% males) and 10 in group B (mean age 60.0 ± 2.5 years, 80% males). Cases of colorectal cancer, IBD and diverticulitis were the comparable in the two groups (Supplementary Table S2). Surgery had been performed 30–40 days previously in all patients.

Three saline-treated patients dropped out, two for the onset of severe ulcerative proctitis treated with steroids. At the end of the trial 7 patients in group B completed the study.

3.1. Endoscopic and histopathological grading

The endoscopic grading was significantly reduced in group A after treatment (from 1.3 ± 0.21 to 0.4 ± 0.16, $p = 0.0083$, $n = 10$) whereas it was unchanged in group B (from 1.37 ± 0.26 to 0.71 ± 0.42, $p = 0.13$, $n = 7$; means ± SE, Fig. 1).

In group A, histopathological grade varied from 1.33 ± 0.3 to 1.67 ± 0.6 after treatment ($p = 0.41$) and from 1.67 ± 0.6 to 3.0 ± 0.0 in group B; $p = 0.34$; means ± SE. However, atrophy was reduced or unchanged in all butyrate-treated patients; it was increased by 42.8% in saline-treated patients, but this difference did not reach statistical significance, Fig. 2.

Table 1
Baseline characteristics of the patients who completed the trial.

	Group A ($n = 10$)	Group B ($n = 7$)
Mean age	60.2 ± 16.6	60.6 ± 9.2
Male gender	8 (80%)	5 (71.4%)
Disease		
IBD	3 (30%)	1 (14.3%)
Diverticulitis	2 (20%)	2 (28.5%)
Carcinoma	7 (70%)	4 (57.1%)
Type of surgery		
Left colectomy and proctectomy with colo-anal-anastomosis, ileostomy	6	3
Left colectomy and RAR with colo-anal anastomosis, ileostomy[a]	1	
Left colectomy with colorectal anastomosis, ileostomy[b]	1	1
Total colectomy with closure of rectal stump, ileostomy	1	1
Total colectomy and ileo-rectal anastomosis, ileostomy	1	1
Hartmann procedure		1
Endoscopic score at baseline	1.30 ± 0.21	1.38 ± 0.25
Histologic score at baseline	1.33 ± 0.3	1.67 ± 0.7

IBD, inflammatory bowel disease.
[a] Anastomosis between colon and anus following total resection of the rectum.
[b] Anastomosis between colon and middle/low rectum after partial resection of the rectum.

3.2. Biosafety of topical butyrate treatment

The serum levels of urea, creatinine, alanine transaminase and γ-glutamyl transferase were within the normal range in both groups after therapy. No local or systemic adverse events were reported.

3.3. SCFA

SCFA determination from biopsies is intrinsically problematic due to the low quantity of tissue available and the quality of the samples. We selected a specific fibre in SPME, comparing various commercially available fibres (Polydimethylsiloxane, 7 μg (PDMS), Carboxen-PDMS, Carbowax-Divinylbenzene (CW/DVB) and Polyacrylate). Our experiments showed that the Carboxen/PDMS fibre was the most sensitive.

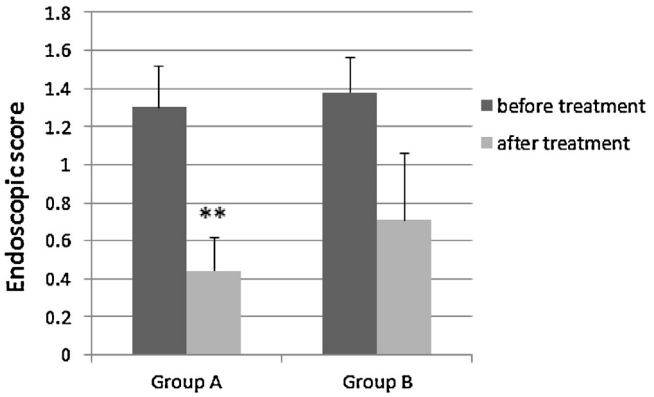

Fig. 1. Endoscopic scores in Group A, receiving enemas containing sodium butyrate (2 g/30 ml) twice daily for 30 days ($n = 10$) and group B, receiving the same volume of saline ($n = 7$) for 30 days, at the beginning and at the end of the treatment. Values are means ± SEM; **$p < 0.01$ vs. baseline.

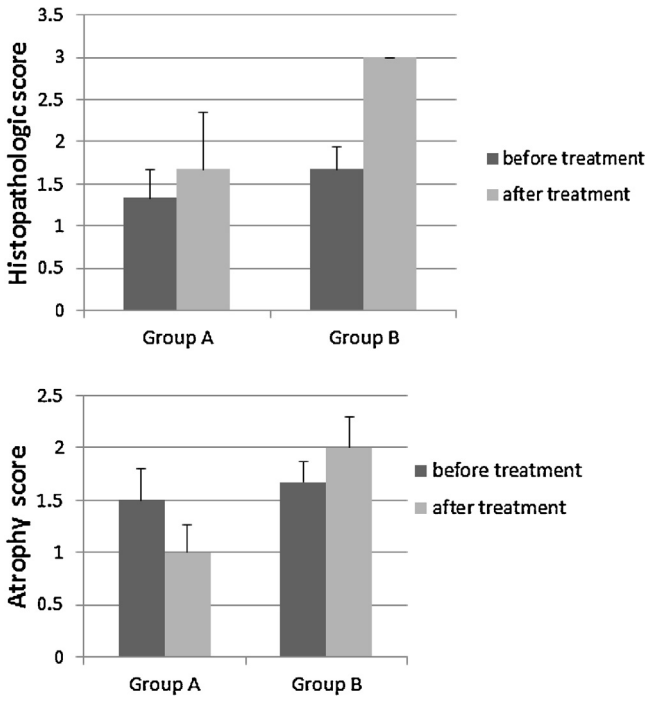

Fig. 2. Panel a: Histopathological score; panel b: Mucosal atrophy score in Group A (butyrate patients, $n = 10$) and group B (saline patients, $n = 7$), at the beginning and at the end of the treatment. Values are means ± SEM. Experimental conditions as in Fig. 1.

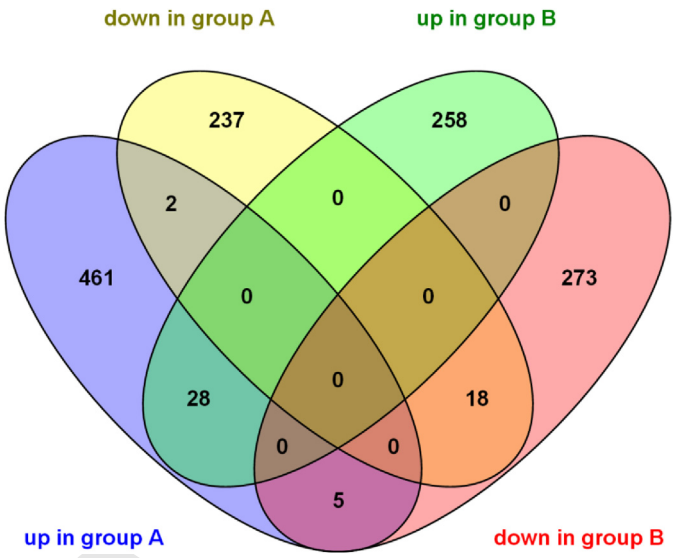

Fig. 3. Venn diagram showing the overlap among genes found differentially expressed after treatment in group A (butyrate patients) and in group B (saline patients). "Up" and "down" indicates up-regulated or down-regulated genes after treatment.

Preliminary analyses on human and rodent colorectal biopsies found levels of propionic acid ranged from 0.051 to 3.13 ng/μg, butyric acid from 0.06 to 2.2 ng/μg and valeric acid from 0.08 to 0.65 ng/μg. The highest amount of butyrate was found in rodents treated with a prebiotic-rich diet and in specimens from human cecum where the amount of butyrate was 124.6 ± 12.26 ng/mg of tissue (mean of 4 biopsies) Nevertheless, no biopsy had SCFA levels above the detection limit.

3.4. Transcriptomic analysis

One out of 13 hybridizations did not pass the quality control criteria and was ignored. In the remaining 12 hybridizations, statistical analyses were performed on the 12,525 genes present in at least 50% of the experiments. Overall, we observed a great variability in the expression profiles in both groups. We found 778 genes differentially modulated by butyrate treatment and 595 by saline administration; only few of them were up- or down-regulated in both groups (Fig. 3).

GO-elite software was used to identify the biological process modulated by each treatment: in group B, saline treatment down-regulated oxidative phosphorylation and AMPK signalling whereas in the butyrate group we found a positive modulation of genes of cell cycle, glutathione metabolism and focal adhesion pathways (Supplementary Table S3).

GSEA was used to identify KEGG pathways that had more differentially expressed genes than expected by chance, by comparing group A to group B. Eleven out of 230 investigated gene sets passed the 0.005 significance threshold using the LS/KS permutation test (Supplementary Table S4, Supplementary Fig. S1), including focal adhesion, regulation of actin cytoskeleton, Toll-like receptor signalling, cell communication and Wnt signalling pathways.

SAM identified 63 genes discriminating between butyrate and saline groups (Supplementary Table S5, Supplementary Fig. S2). The most differentially expressed gene was the *Homo sapiens gremlin 2* (*GREM2*) that was 11.45 times more expressed in group A than group B patients (mean 9.02-fold change in butyrate- and 0.79-fold in saline-patients). We noted other genes belonging to the Bone morphogenetic protein (BMP) antagonist family, such as *GREM2* and *SOSTDC1* significantly modulated by butyrate treatment (Supplementary Table S6). To explore this aspect, we analyzed a panel of BMP antagonists in all patients enrolled in the study by RT-PCR.

3.5. RT-PCR

The expression of 6 BMP antagonists was analyzed by semi-quantitative RT-PCR in the 17 patients who had completed the trial, at day 1 and day 30. Overall we observed an increase in the expression of BMP antagonists after butyrate therapy that was statistically significant for *GREM1* ($p < 0.05$) and *SOSTDC1* ($p < 0.05$) (Fig. 4). If we separate data on patients having CRC, IBD or diverticulitis the increase in gremlins expression after butyrate treatment was mainly observed in IBD patients (Fig. 5).

4. Discussion

A colon diversion is generally created to protect a distal anastomosis from the faecal stream after colorectal surgery. The most common complication of this procedure is diversion colitis, an inflammatory and dystrophic disease of the lining of the large intestine, probably related to the absence of faecal transit and reduced availability of energy substrates for endoluminal colonocytes. This condition may alter the colon with such severe inflammation and ulceration that a stricture occurs which requires removal [13].

It has been speculated that the absence of faecal transit causes a reduction in the levels of SCFAs in the mucosa, making mucosal cells unable to metabolize them as an energy source for the proper tropism. The importance of a good cell nutrition is evident as studies show that the restoration of intestinal transit, the normalization of the supply of SCFAs, or the administration of enemas rich in SCFAs into the excluded segments can reverse the inflammatory process [11,13].

The use of SCFAs in patients with diversion colitis was attempted before in few studies using concentrations ranging from 15.6 to

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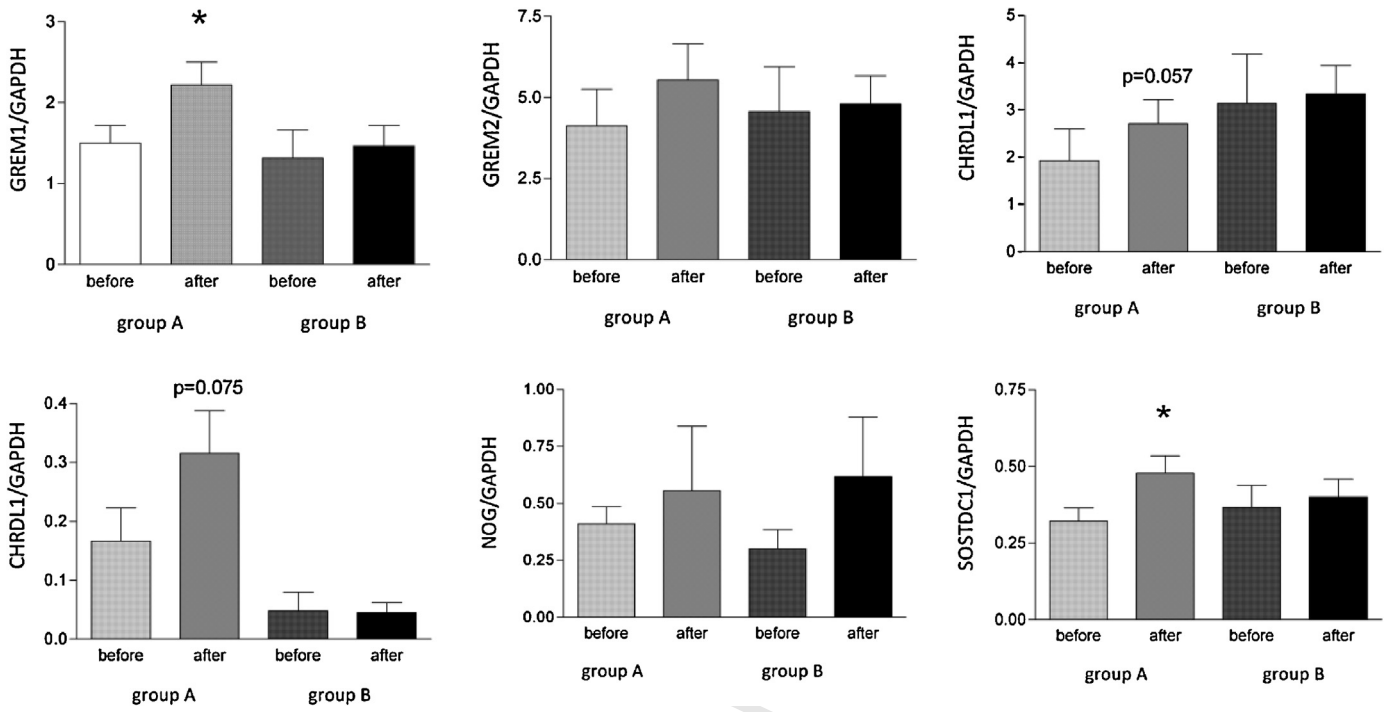

Fig. 4. Expression of bone morphogenetic protein (BMP) inhibitors in Group A (butyrate-treated patients, $n = 10$) and group B (saline-treated patients, $n = 7$), at the beginning and at the end of the treatment, expressed as ratio of each gene mRNA to the GAPDH mRNA, co-amplified. Each column represents the mean ± SEM. *$p < 0.05$, vs. baseline.

30 mM daily [10–12], comparable to the 36 mM daily butyrate used in the present work. However, despite the relatively high concentration of butyrate used here, butyrate was not detectable in rectal or colon biopsies, suggesting its rapid use by colonic epithelial cells. We did not observe any adverse effects due to the treatment; butyrate is in fact a physiological substrate present at high concentration in the colon of mammals and no toxic effects have been reported following butyrate enemas [10–12] or intravenous administration in leukaemia (500 mg/kg/d for 10 days) [14].

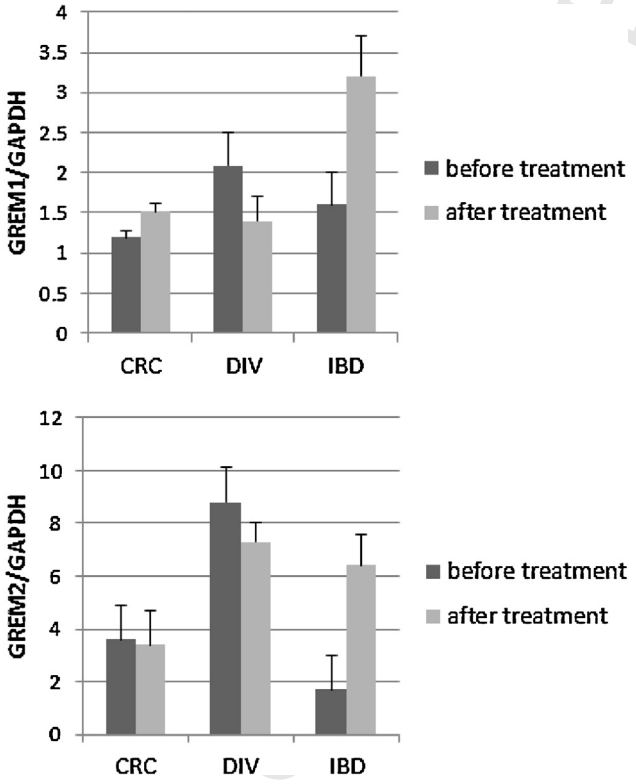

Fig. 5. Relative expression of *GREM1* and *GREM2* genes in Group A (butyrate-treated patients), in colorectal cancer ($n = 5$), diverticulitis ($n = 2$), and inflammatory bowel disease cases ($n = 3$), before and after treatment. Means ± SEM. CRC, colorectal cancer; DIV, diverticulitis; IBD, inflammatory bowel disease.

Our results demonstrate a positive effect of post-operative butyrate administration on colonic anastomosis. A 30-day long treatment improved the endoscopic score of butyrate-treated patients and controlled mucosal atrophy. On the contrary, despite a slight improvement in endoscopic scores, the colon mucosa of patients receiving saline exhibited signs of impaired colonic homeostasis. Transcriptomic analysis of group B patients showed in fact a down-regulation of genes associated with oxidative phosphorylation and an increased expression of genes involved in the AMPK signalling pathways.

It has been reported that germ-free murine colonocytes are in an energy-deprived state showing a marked decrease in NADH/NAD(+) ratio, oxidative phosphorylation and ATP levels which result in AMPK activation and butyrate supplementation is able to rescue their mitochondrial respiration deficiency [15].

GSEA analysis identified a number of biological processes associated with mucosal repair after butyrate treatment such as cell communication, Toll-like receptor signalling pathway, focal adhesion, Wnt signalling pathway, regulation of actin cytoskeleton and adherent junction.

The communication among epithelial cells, sub-epithelial lamina-propria cells, including myofibroblasts, may play a key role in the recovery of epithelial barrier function [16]. The restoration of tissue integrity involves the coordinated interaction of various cell types, the deposition of extracellular matrix, the release of soluble growth factors and the up-regulation of epithelial cell proliferation [17]. Adhesion-mediated signalling between cells and the matrix plays a critical role in maintaining tissue homeostasis as well as in the response to tissue damage [17]. Several growth factors are potent stimulators of epithelial cell migration including transforming growth factor (TGF)-β and insulin-like growth factor [18], both significantly up-regulated in butyrate patients. Another group of

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compounds that plays a well-established role in epithelial restitution are the polyamines, putrescine, spermidine, spermine, [19], also up-regulated after butyrate treatment, together with ornithine decarboxylase and spermidine synthase.

The absence of enteral nutrition leads to intestinal mucosal atrophy; numerous mechanisms may modulate such changes in intestinal homeostasis, including variations in genes of the BMP family and the Wnt-β-catenin signalling pathways. The gene expression profiles of the biopsies harvested from butyrate patients show an up-regulation of Wnt signalling and of BMP antagonists such as *GREM1* and *SOSTDC1* regulating BMP signalling through direct interaction with BMP ligands, thereby blocking ligand–receptor interaction [20].

Wnt signalling is involved in the self-renewal of stem cells and evidence suggests that the BMP pathway provides pro-differentiation cues that serve as a counterbalance to Wnt-induced proliferation [21–23].

In murine and human colon, it has been demonstrated that BMP2 and its receptors are highly expressed on surface colonocytes which are mature cells that are no longer proliferating and will soon undergo apoptosis and/or be shed into the gut lumen. I*n vitro*, BMP2 acts to inhibit proliferation and promote apoptosis, increasing cleaved caspase 3 and β-catenin expression, and decreasing expression of the cyclin PCNA [24]. Remarkably, in a mice model of total parenteral nutrition, an increase in *BMP2*, *BMP4*, and *BMP type II receptors* at the RNA and protein levels, and a lower expression of *WNT3, WNT5a*, and *the WNT receptor Lrp5*, has been reported, suggesting that the activation of the BMP signalling pathway may be involved in the development of intestinal mucosal atrophy [25]. On the contrary, over-expression of the *NOG* antagonist leads to an increased number of crypts in ectopic locations, likely via increased nuclear β-catenin in the stem cell compartment from cross-talk between the BMP and Wnt pathways [26,27]. Barker and Clevers recently pointed out the similarity between BMP antagonists such as the gremlin, and ligands of the Drosophila receptor Lgr2, the fly orthologue of mammalian Lgr4, Lgr5, and Lgr6, orphan G-protein-coupled receptors, markers of stem cells in the intestine [28].

The up-regulation of BMP antagonists, especially *GREM1*, after butyrate enemas, suggests that the beneficial effects of such treatment can help restore mucosal integrity postoperatively. When we subdivided butyrate-treated patients according to their disease, we observed that the up-regulation of *GREM1* and 2 was mostly observed in patients suffering from IBDs. The role of the BMP signalling in IBDs has recently been pointed out and appears to be complex. BMP signalling is essential for the inflammatory response of vascular endothelial cells [29] and BMP2 and BMP4, secreted from infiltrating inflammatory cells, have been shown to activate BMP signalling in the surface epithelial cells of gastric mucosa from individuals with *Helicobacter pylori* infection [30]. Wang and co-workers have tested anti-BMP agents in murine models of intestinal inflammation, observing a significant reduction in colon Il17 expression [31].

A number of recently published papers report a loss of butyrate-producing bacteria in IBD patients suggesting that reduced butyrate levels may contribute to the pathogenesis of such diseases [32–34] and that these patients may benefit from exogenous administration of butyrate.

In conclusion, our results, although in a limited number of patients, suggest that butyrate enemas may help to restore the integrity of the colorectal mucosa after surgery, especially in IBD patients, and reveal the extreme complexity of the signalling networks in intestinal epithelium dynamics.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dld.2015.09.005.

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