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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 ng/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.