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Cellular changes of the colon after mechanical bowel preparation

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ARTICLE INFO

Article history:
Received 9 June 2013
Received in revised form
13 August 2014
Accepted 21 August 2014
Available online 27 August 2014

Keywords:
Mechanical bowel preparation
Butyrate
Colonocyte

ABSTRACT

Background: The purpose of this study was to evaluate the effect of mechanical bowel preparation (MBP) on the intracellular environment, specifically evaluating butyrate transport, within the colon of the Sprague–Dawley rat.

Methods: Sixty-eight Sprague–Dawley rats were randomized to either an MBP group ($n = 34$) or a control group ($n = 34$). Twenty-four hours after the completion of the MBP, both groups were euthanized, and the colons were harvested. The level of cellular apoptosis was investigated after DNA fragmentation, poly(ADP-ribose) polymerase cleavage, and caspase assays. Western blot analysis was performed to measure the expression of the butyrate transporter protein, monocarboxylate transporters 1, and proliferating cell nuclear antigen (a marker for tissue proliferation). Immunohistochemical staining was performed to further investigate cellular proliferation. Statistical significance ($P < 0.05$) was determined using two-tailed t-test.

Results: Apoptosis was detected without significant differences in both groups. Western Blot analysis demonstrated that the expression of the monocarboxylate transporters 1 protein is downregulated in the MBP group (10.18 ± 3.09) compared with the control group (16.73 ± 7.39, $P = 0.001$), and proliferating cell nuclear antigen levels showed a decrease in cellular proliferation in the MBP group (13.35 ± 5.88) compared with the control (20.07 ± 7.55, $P = 0.018$). Immunohistochemistry confirmed a decrease in cellular proliferation after MBP with 23.4 ± 7.8% of the cells staining positive for Ki-67 in the MBP group *versus* 28.6 ± 7.9% in the control group ($P = 0.006$).

Conclusions: MBP has a negative impact on cellular proliferation and intracellular transport of butyrate within the rat colon, not related to apoptosis. This is the first study to demonstrate the intracellular effects that MBP has on the rat colon.

Published by Elsevier Inc.

This project was presented as a Podium Presentation in the Colorectal Surgical forum at the American College of Surgeons meeting, Chicago IL, October 6–10, 2012.

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0022-4804/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.jss.2014.08.035

1. Introduction

Patients with diseases of the colon and rectum often require mechanical bowel preparation (MBP) before endoscopic procedures and surgical interventions. The commonly used regimen for bowel preparations includes sodium phosphate containing solutions or polyethylene glycol (PEG). PEG-based preparations, an osmotically balanced electrolyte lavage solution, are often considered the gold standard [1]. Despite the widespread use, several prospective trials and meta-analysis have now demonstrated the feasibility and safety of elective colorectal procedures without MBP [2–4]. Furthermore, some evidence has shown that MBP may in fact increase morbidity, most concerning of which is an increase in the rate of anastomotic leaks, and wound infections [5]. Although it remains unclear by what mechanism MBP may result in increased complication rates, MBP has been associated with subtle structural changes to the colon to include mucin depletion, loss of epithelial cells, and inflammation [6].

n-Butyrate, the natural fuel source for colonic mucosa, has become the subject of several recent investigations. In addition to being an important respiratory fuel, butyrate is involved in cellular maturation and differentiation [7]. Butyrate is also integral to mucin production and reepithelization of the colonic mucosa. Interestingly, recent evidence has demonstrated that supplemental butyrate facilitates anastomotic healing in both healthy and radiated tissue; an important finding that is clinically relevant to intestinal surgery [8]. The effect that MBP may have on the utilization of butyrate within the colon has not been investigated.

Although the histologic effects of MBP have been investigated, it is unknown what alterations may take place at the cellular level. The purpose of this study was to evaluate the effect of MBP on the intracellular environment, by evaluating cellular proliferation, butyrate transport, and apoptosis within the colon of the Sprague–Dawley rat.

2. Methods

2.1. Animal model

The Institutional Animal Care and Use Committee of William Beaumont Army Medical Center approved this study. The rats (Harlan Labs, Houston, TX) were cared in accordance with the guidelines of National Institutes of Health for care and use of laboratory animals. Sixty-eight male Sprague–Dawley rats (250–275 g) were housed in individual metabolic cages. The rats were randomly divided into an MBP group ($n = 34$) and a control group ($n = 34$). On the day before tissue harvest, the MBP group received PEG water 5.9% solution via gastric lavage, followed by food restriction. The control group was provided standard food *ad libitum*, Purina Lab Diet 5001 (PMI Nutrition International, St. Louis, MO). Both groups were provided with plain reverse osmosis water *ad libitum*, via an automatic watering system.

2.2. Tissue procurement

On the day of tissue harvest, the animals were sedated before euthanasia with pentobarbital sodium, 40 mg/kg intraperitoneal. Euthanasia was performed via intravenous injection of Beuthanasia solution (0.2 mL/kg). Abdominal access was achieved by a midline laparotomy incision postmortem. The cecum and ascending colon were freed of the mesentery, and the small bowel and transverse colon were ligated with 2-0 silk sutures and removed. The colon was then incised longitudinally along the antimesenteric border exposing the lumen and contents. Two specimens of intact, full thickness colon were collected from each animal. One was placed in formalin for immunohistochemical staining, whereas the other was snap frozen in liquid nitrogen and stored at $-70°$ for further analysis.

2.3. Tissue homogenization

Colonic tissues were homogenized in five volumes of tissue protein extraction reagent containing protease and phosphatase inhibitors, according to the manufacturer's protocol (Thermo Scientific, Rockford, IL). After centrifugation at $12{,}000 \times g$, protein content in the supernatant was determined using the Bradford Protein Assay Kit (Thermo Scientific). The homogenate was subsequently used for the analysis of free-fatty acids (FFA), caspase activity assay, and Western blotting of proliferating cell nuclear antigen (PCNA) and Monocarboxylate transporters 1 (MCT-1).

2.4. Measurement of FFA in colonic tissue lysate and serum

The tissue extract and serum was used to assay FFA using a Zenbio enzyme-linked immunosorbent assay kit: Serum/Plasma Non-Esterfied Fatty Acids Detection 500 Point Kit according to the manufacturer's protocol. Plates were read at 540 nm. Protein determinations were determined using Coomassie Plus protein assay reagent (Thermo Scientific). Protein levels were expressed in microgram per milliliter, and FFA values were converted to micromole FFA per microgram protein.

2.5. Western blot analysis for PCNA

Equal amounts of protein (20 μg) was loaded into each well and resolved by electrophoresis on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to a nitrocellulose membrane using a wet electrophoretic transfer apparatus. The membrane was incubated in blocking buffer (5% nonfat dry milk in PBS-Tween 20, 0.1% [vol/vol]) for 3 h at room temperature. The membrane was incubated at room temperature with a specific PCNA monoclonal antibody (mAb) (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:200 for 4 h. The membrane was then washed in PBS-Tween 20 four times, 15 min each, and then incubated in diluted (1:20,000) IRDye800-conjugated anti-mouse secondary antibody for 30 min at room temperature. The membrane was again washed four times for 15 min each in PBS-Tween 20. Electronic image of the blot was captured using the Odyssey Infrared Imaging system (LI-COR Biosciences, Lincoln, NE). The relative intensities were quantified by Li-COR densitometric analysis software. Equal loading of the protein was verified by Western blotting the membrane with

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