Down-Regulation of the Monocarboxylate Transporter 1 Is Involved in Butyrate Deficiency During Intestinal Inflammation

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Background & Aims: **Butyrate oxidation is impaired in intestinal mucosa of patients with inflammatory bowel diseases (IBD). Butyrate uptake by colonocytes involves the monocarboxylate transporter (MCT) 1. We aimed to investigate the role of MCT1 in butyrate oxidation deficiency during colonic inflammation.** ***Methods:*** **Colonic tissues were collected from patients with IBD or healthy controls and from rats with dextran sulfate sodium (DSS)-induced colitis. The intestinal epithelial cell line HT-29 was treated with interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). MCT1 expression was analyzed by real-time reverse-transcription polymerase chain reaction, Western blot, and immunofluorescence. Butyrate uptake and oxidation in HT-29 cells was assessed using [^{14}C]-butyrate. The mechanism of MCT1 gene regulation was analyzed by nuclear run-on and reporter gene assays.** ***Results:*** **MCT1 messenger RNA (mRNA) and protein levels were markedly decreased in inflamed colonic mucosa of IBD patients and rats. In HT-29 cells, down-regulation of MCT1 mRNA and protein abundance by IFN-γ and TNF-α correlated with a decrease in butyrate uptake and subsequent oxidation. IFN-γ and TNF-α did not affect MCT1 mRNA stability but rather down-regulated gene transcription. We demonstrate that the cytokine response element is located in the proximal −111/+213 core region of the MCT1 promoter.** ***Conclusions:*** **The data suggest that butyrate oxidation deficiency in intestinal inflammation is a consequence of reduction in MCT1-mediated butyrate uptake. This reinforces the proposition that butyrate oxidation deficiency in IBD is not a primary defect.**

Butyrate is a short-chain fatty acid produced by colonic bacterial fermentation of dietary fibers. Butyrate plays an important part in maintaining the health and integrity of the colonic mucosa. It is the primary energy source for the colonic epithelium[1] and regulates cell proliferation,[2] differentiation, and apoptosis.[3] In addition, butyrate has an important role in modulating mucosal inflammation.[4,5]

Ulcerative colitis (UC) and Crohn's disease (CD) are the 2 major inflammatory bowel diseases (IBD). Several studies have found that butyrate oxidation is decreased in the inflamed mucosa of patients suffering from UC[6,7] or CD[8] and in animal models of experimental colitis.[9] However, these studies have suggested that butyrate oxidation deficiency is not a primary defect. Indeed, butyrate oxidation is impaired in patients with active UC but not with quiescent UC.[7] Also, in mice with dextran sulfate sodium (DSS)-induced colitis, butyrate oxidation was impaired only after 6 days of DSS treatment.[9] These studies did not consider a potential reduction in butyrate uptake by intestinal epithelial cells as a causative factor of this butyrate oxidation deficiency.

The human monocarboxylate transporter 1 (MCT1) gene encodes for a plasma membrane protein of 45 kilodaltons containing 12 α-helical transmembrane domains with C- and N-termini located within the cytoplasm.[10] We and others have demonstrated that MCT1 transports butyrate across the apical membrane of human colonocytes.[11,12] Thus, a decrease in MCT1 expression, which reduces the intracellular availability of butyrate,[13] could affect not only its oxidation but also its cell regulatory effects. Indeed, we have shown that silencing MCT1 expression by RNA interference in colonic epithelial cells decreases butyrate induction of cell-cycle arrest and differentiation.[13] This finding suggests that a decrease in MCT1 expression could negatively affect colonic tissue homeostasis.

In human colonic tissues, during transition from normality to malignancy, decreased MCT1 expression has been reported.[14] However, very little is known about

Abbreviations used in this paper: CAPE, caffeic acid phenylethyl ester; DSS, dextran sulfate sodium; IFN, interferon; MCT, monocarboxylate transporter; MTT, [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; NF-κB, nuclear factor-κB; TNF, tumor necrosis factor.

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MCT1 expression in IBD. We hypothesize that the impairment in butyrate oxidation reported in active IBD could be related to a decrease in MCT1 expression in the inflamed colonic mucosa. We observed that inflammation caused down-regulation of MCT1 expression in the colonic tissue. Furthermore, treatment of intestinal epithelial cell lines with proinflammatory cytokines induced down-regulation of MCT1 expression that was associated with a reduction in butyrate uptake and subsequent oxidation. Therefore, butyrate oxidation deficiency in intestinal inflammation appears to be a consequence of reduction in MCT1-mediated butyrate uptake and sustains the idea that butyrate oxidation deficiency in IBD is not a primary defect.

Materials and Methods

Patients and Biopsies

Colonic biopsy specimens were obtained from inflamed and noninflamed mucosa of 14 patients with CD (9 women, 5 men; mean age, 36 years; range, 21–78 years) and 9 patients with UC (4 women, 5 men; mean age, 46 years; range, 30–62 years). All patients underwent colonoscopy for an active disease. At the time of the study, 9 patients were receiving steroids; 2 patients, 5-aminosalicylic acid; 2 patients, azathioprine; 1 patient, 6-mercaptopurin plus infliximab; 1 patient, cyclosporin; and 8 patients, no medication. Colonic biopsy specimens were collected from healthy mucosa of 10 asymptomatic subjects undergoing routine colonoscopy. The study was approved by the "Fédération des Biothèques" of the University Hospital, Nantes. All patients gave informed consent to take part in the study.

Induction of Colitis in Rats

Principles of laboratory animal care and guidelines according to the Declaration of Helsinki were followed. Sprague-Dawley male rats were treated with 4% DSS in drinking water or with water alone (control) for 5 days. Rats were then killed and colonic segments removed for RNA or protein isolation.

Cell Culture and RNA Interference

The parental intestinal epithelial cell line HT-29 was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 μg/mL streptomycin, 100 IU/mL penicillin, and 2 mmol/L glutamine. Cells were treated with interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) at the indicated time and doses. Cell viability was assessed using the [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) viability assay (Promega, Charbonnieres, France). To study the effect of IFN-γ and TNF-α on the stability of the MCT1 transcript, cells were stimulated in the presence of 1 μg/mL actinomycin D (Sigma) for 2, 4, 6, 8, and 24 hours. In other experiments, cells were preincubated for 1 hour with 10 μmol/L of the nuclear factor (NF)-κB inhibitor caffeic acid phenylethyl ester (CAPE) (Calbiochem, Nottingham, UK) before treatment with cytokines as described above.

Transfection and Luciferase Assays

pGL3-basic luciferase reporter plasmids containing successive deletions of the 5′-end of the MCT1 promoter (−1525, −1319, −1106, −896, −703, −476, −307, −111) were used to assess promoter activity, as previously described.[15] HT-29 cells were transfected in 96-well plates with 250 ng pGL3-MCT1 plasmid construct or pNFκB-Luc vector (Clontech, Saint-Germain-en-Laye, France) and 100 ng pIRES-EGFP vector (internal control; Clontech) using Lipofectamine 2000 (Invitrogen). Twenty-four hours later, cells were stimulated with increasing doses of TNF-α and IFN-γ for 24 hours. Cell lysates were assayed for luciferase activity using the firefly luciferase 1-step assay kit (Fluoprobes, Montlucon Cedex, France). Luminescence and fluorescence were then measured with a luminometer/fluorimeter (VICTOR[3], PerkinElmer, Courtaboeuf Cedex, France). Luciferase activity was normalized to fluorescence intensity and expressed as relative light units (RLU).

Real-Time Reverse-Transcription Polymerase Chain Reaction

Total RNA was isolated with TRIzol reagent (Invitrogen, Cergy Pontoise Cedex, France) and treated for 45 minutes at 37°C with 2 U RQ1 DNAse (Promega). One microgram RNA was reverse transcribed using Superscript III Reverse Transcriptase (Invitrogen). One microliter of the complementary DNA (cDNA) solution was subjected to real-time quantitative polymerase chain reaction (PCR) in a Bio-Rad iCycler iQ system using the QuantiTect SYBR Green PCR kit (Qiagen, Courtaboeuf Cedex, France). Quantitative PCR consisted of 45 cycles, each PCR cycle consisting of 30 seconds at 95°C and 30 seconds at 60°C. The sequences of human and rat primers for MCT1, interleukin (IL)-1β, β2-microglobulin, and β-actin are included in Supplementary Table 1 (see Supplementary Table 1 online at www.gastrojournal.org). The expression level of β-actin was used as a reference value to normalize MCT1 and IL-1β gene expression. Relative quantitative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method,[16] using normal tissues from healthy subjects, control rats, or untreated control cells as the calibrator samples.

Western Blot Analysis

Membrane protein samples were either from HT-29 postnuclear membranes, prepared as previously described,[17] or from patient biopsy specimens and rat colonic tissues. The latter were coextracted with RNA using TRIzol reagent.