Dig Dis Sci (2013) 58:3386–3388
DOI 10.1007/s10620-013-2840-5

EDITORIAL

Energy, Oxidative Stress, and Inflammation in the Colon

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Published online: 25 August 2013

The manuscript by Martinez et al. [1] describes the effects of topically applied *N*-acetylcysteine (NAC) on colonic histology and oxidative DNA damage in a rodent model of diversion colitis. The antioxidant NAC significantly improved histological inflammation scores in the diverted colon segments after 2 and 4 weeks of treatment. Furthermore, oxidative DNA damage in colonocytes was significantly reduced in segments without and with fecal stream exposure. These observations broaden our understanding of the role of oxidative stress in diversion colitis, and provide an opportunity to examine other interrelated factors involved in inflammation of the colon.

Energy metabolism in colonocytes is dependent on the luminal availability of short-chain fatty acids (SCFA), for example butyrate, propionate, and acetate [2]. Obligate anaerobic bacteria within the gut microbiome ferment and break down dietary complex carbohydrates and proteins to produce SCFA. Whereas glucose is a principal energy source for enterocytes in the small intestine, colonocytes are dependent on SCFAs to maintain energy homeostasis. Experimental and clinical studies support the concept that luminal deficiency of SCFAs, for example butyrate, exacerbate diversion colitis [3–5]. Clinical trials utilizing SCFA enemas to treat diversion colitis have been limited and inconclusive. Thus, because SCFA enemas are not used routinely to treat diversion colitis, surgical restoration of bowel continuity is definitive [5].

Butyrate regulation of energy metabolism in colonocytes has been studied in germfree (GF) mice [6]. Similar to humans, mouse colonocytes utilize bacterially-produced butyrate as their primary energy source. In conventionally raised mice, butyrate is required for normal colonocyte ATP concentration. When gut flora is normal, microbiome-generated butyrate is transported into colonocytes, entering the mitochondria, after which butyrate undergoes β-oxidation to acetyl-CoA, which then enters the tricarboxylic acid (TCA) cycle, reducing NAD+ to NADH. Upon entering the electron transport chain, NADH generates ATP and CO_2. In the GF state, or likely in colonic diversion, colonocytes increase glucose uptake which increases the rate of glycolysis and lactate production at the expense of oxidative metabolism. Consequently, colonocyte ATP concentration is reduced because of inadequate butyrate availability. Nutrient-deficient and energy-deficient colonocytes will experience alterations in their redox state and decreased cellular oxidative phosphorylation with resultant increase in oxidative stress. In an effort to maintain energy homeostasis and cellular integrity, colonocytes in GF mice degrade cellular proteins and damaged organelles to generate amino acids for energy consumption in a process termed autophagy [6]. Addition of butyrate to GF colonocytes partially restores mitochondrial respiration and prevents autophagy. If these rescue efforts, for example autophagy, are not successful then cell death or apoptosis occurs. Recent research indicates that the process of autophagy is also important for clearing intracellular pathogens and may be an important component in immune surveillance and innate and adaptive gut immunity [7].

The ability of topically delivered NAC to ameliorate colitis in colon segments diverted from luminal bacteria and nutrients draws attention to the role of oxidative stress in the perpetuation of this pathologic condition. NAC is a water-soluble amino acid with L-cysteine and one acetyl group. The hydrogen atom in the sulfhydryl (–SH) of sulfur-containing antioxidant molecules (thiols), including NAC and

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glutathione (GSH), acts as an electron source for neutralizing or reducing free radicals which contain one or more unpaired electrons on either oxygen (reactive oxygen species, ROS), or nitrogen (reactive nitrogen species, RNS) [8, 9]. Importantly, NAC also provides L-cysteine for synthesis of glutathione, an endogenous cellular antioxidant. Examples of oxygen-derived free radicals include superoxide ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2); examples of nitrogen free radicals are peroxynitrite ($ONOO^-$) and nitric oxide ($NO^{\cdot}$). Free radicals are normally produced at low levels during cellular metabolism, particularly in the mitochondria, are short-lived, and are hydrogen-reduced by endogenous antioxidants, for example glutathione. Free radicals such as hydrogen peroxide and nitric oxide, produced enzymatically under stable conditions, mediate diverse physiological functions through cell signaling. Under conditions of oxidative stress, mitochondrial glutathione concentrations are diminished, increasing free radical production, inducing organelle dysfunction, autophagy, and resultant cytotoxicity [10].

Inflammation of the colon in experimental and clinical diversion colitis is characterized histologically by mucosal ulceration, crypt distortion, lamina propria infiltration by neutrophils, lymphocytes plasma cells and lymphoid hyperplasia [1, 4].

Appreciation of factors involved with initiation and perpetuation of inflammation in diversion colitis may relate, in part, to some of the issues previously mentioned, for example energy deprivation and oxidative stress. These factors may have implications to other forms of bowel inflammation.

Butyrate metabolism by mitochondria, under stable in-vitro conditions, generates low levels of ROS which, indirectly, inhibit the nuclear transcription pathway involving NF-κB [11]. Alternatively, the energy defect encountered in diversion colitis can lead to oxidative stress and high ROS concentrations, stimulating NF-κB signaling, DNA binding, and subsequently, inflammatory cytokines production [10]. Mild increases in ROS stimulate production of another transcription factor, Nrf2, which translocates to the nucleus where it binds to the antioxidant response elements (ARE) present on stress responsive genes [10]. In turn, Nrf2 binding to ARE activates genes involved in cellular antioxidant and anti-inflammatory defense mechanisms. Excessive ROS concentrations and oxidative stress interfere with Nrf2 translocation, enhancing NF-κB signaling in an effort to protect the cells. This concentration and duration effect of biological substances, for example ROS and signaling proteins, is indicative of the duality of their potential actions and the importance of the regulatory systems needed to maintain homeostasis in cells and tissues.

Martinez and colleagues have also reported that 5-aminosalicylic acid (5-ASA) enemas in rats with diversion colitis effectively reduced microscopic mucosal injury and inflammation, and reduced oxidative DNA damage [12]. Moreover, 5-ASA functions as an antioxidant, a prostaglandin synthesis inhibitor, and a modulator of inflammatory cytokine production via the NF-κB pathway. Mesalamine, as topical or oral therapy, is a durable mainstay in frontline therapy for ulcerative colitis when formulated to deliver 5-ASA to sites of colonic inflammation. Because NAC and 5-ASA are pharmacologically similar, if one antioxidant is good might two be better in treating colitis? Combining the two antioxidants for topical delivery to the inflamed distal colon in a rodent model of colitis significantly and synergistically improves histological scores [13]. Moreover, simultaneous administration improved inflammatory biomarkers significantly more than with either agent used alone. Validation of the concept that the combination of NAC and 5-ASA is better than one for treating distal ulcerative colitis is now being addressed in clinical trials.

Targeted antioxidant therapy to specific detrimental reactive species at critical sites in a given disease state is an approach that has merit for future research. The targeted, topical delivery of NAC to the site of inflammation in diversion colitis may be one positive step in that direction. The complexities and binary functions of reactive species and transcription factors, for example, pose challenges for the investigator to find novel and effective approaches to diseases that develop as consequences of alterations in cellular energy, oxidative stress, and inflammation.

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