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Research Article

Diversion Colitis: A Bioenergetic Model of Pathogenesis

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1. Abstract

Diversion colitis is characterized by mucosal inflammation in segments of the colon that are surgically diverted from the fecal stream. This inflammatory disorder is reported to occur in up to 100% of individuals after colostomy or ileostomy, often occurring within a year following surgery. The lack of short chain fatty acids such as butyrate in the excluded colonic segment is thought to play a role in development of this illness. The actual mechanism leading to colitis remains a mystery however; a review of bioenergetic alterations as a result of disrupted energy flow in different disease models suggests that compensatory anapleurotic (Krebs cycle) metabolism of glutamine as an energy source leads to a sequestration of this amino acid away from nucleotide and glutathione biosynthesis resulting in mucosal atrophy and diminished colonocyte glutathione respectively. Decreased colonocyte glutathione leads to a subsequent rise in cellular hydrogen peroxide (oxidative stress) that initiates mucosal inflammation (colitis) after diffusing through the cell membrane to the extracellular space. Increased anapleurotic metabolism of glutamine leading to decreased availability for nucleotide and glutathione synthesis is compatible with a causal role in the development of mucosal atrophy and mucosal inflammation characteristic of diversion colitis. The aim of this paper is to detail an evidence-based model of diversion colitis based on colonocyte bioenergetic pathways that can explain pathogenesis and provide a scientific basis for therapy.

2. Keywords: Diversion colitis; Oxidative stress; Glutamine, Hydrogen peroxide, Ileostomy; Colostomy.

3. Introduction

Diversion colitis is a reactive colonic inflammatory response in the by-passed segment of the large intestine as a result of fecal stream diversion secondary to colostomy or ileostomy. The lack of short chain fatty acids (SCFAs) such as butyrate in the defunctioned (excluded) colonic segment is thought to play an important role in development of this type of colitis [1]. The colonic epithelium utilizes butyrate for most of its energy requirements [2]. The normal bioenergetic flow begins with the production of butyrate from the fermentation of dietary soluble fiber by colonic bacteria. Butyrate is rapidly absorbed by colonic epithelial cells via passive diffusion and cell membrane transport proteins [3]. Once in the cytoplasm, butyrate is transported via the carnitine shuttle into mitochondria where it undergoes beta-oxidation**.**The resulting acetyl-CoA enters the Krebs cycle, which generates reducing equivalents (NADH, $FADH_2$) that provide the energy for oxidative phosphorylation and ATP production (**Figure 1a-g**) [4]. This process provides up to 70% of colonocyte energy supplies[5]. Disruption of this critical source of energy gives rise to metabolic adjustments that utilize alternate energy sources as a means to fuel cellular energy requirements.

4. Disease Mechanism

Experimental interventions designed to disrupt this energy flow by inhibition of beta-oxidation (with 2-bromo-octanoate) or inhibition of coenzyme-A formation (secondary to vitamin B-5 deficiency) result in murine and porcine colitis respectively (**Figure 2. d-e**)

[6,7]. Soluble fiber is at the beginning of this pathway and the absence of this fermentable carbohydrate subsequent to colonic by-pass surgery disrupts the flow of energy upstream from both acetyl-CoA and beta-oxidation. Because soluble fiber, beta-oxidation and acetyl-CoA are on the same bioenergetic pathway, it suggests a common mechanism for the development of colitis by interventions that disrupt the flow of energy through this pathway (**Figure**. **2 a-f**).

The colonic bacterial flora produces butyrate (and other SCFAs) used by colonocytes as a metabolic fuel. In the absence of butyrate colonocytes increase their usage of glutamine. Studies on isolated colonocytes from germ free rats (that cannot produce SCFAs) report a 45% increase in glutamine use by these cells compared to conventionally reared animals [8]. Other studies have shown significant improvement in experimental diversion colitis following glutamine enemas in rodents [1, 9]. These results suggest that experimental interventions which disrupt energy flow in the bioenergetic pathway from lumenal soluble fiber through acetyl-CoA lead to colitis and are associated with increased glutamine metabolism (**Figure 2**). It also raises the possibility that glutamine has a role in the chain of events leading to colitis. But why should glutamine, an amino acid, have this effect and why is its usage increased so dramatically by colonocytes in the absence of butyrate?

The answer may lie in bioenergetics. Glutamine is used as an alternate cellular fuel during energy restricted conditions [10].When the flow of energy from soluble fiber via butyrate to the formation of acetyl-CoA is inhibited a compensatory increase in the anapleurotic metabolism of the amino acid glutamine occurs in order to replenish Krebs cycle intermediary metabolites (**figure 2f**) [11]. Glutamine is enzymatically converted to glutamate, which enters the Krebs cycle after being converted to alpha-keto glutarate. However, Glutamine directly supports the biosynthetic needs of cell growth and division. Carbon from glutamine is used for amino acid and fatty acid synthesis while nitrogen from glutamine contributes directly to *de novo* biosynthesis of both purines and pyrimidines [11]. The diversion of glutamine to supply cellular energy requirements restricts its (glutamine's) availability for nucleotide synthesis. Without a steady supply of nucleotides, the colonic epithelium (which undergoes continual renewal) cannot sustain its high mitotic index (DNA replication) and turnover (cell division) rate. Under these circumstances the epithelium cannot produce new cells and appears microscopically atrophic with shortened epithelial height [12]. Mucosal atrophy is a histopathological characteristic of diversion colitis. Being a precursor of glutamate, glutamine is also required for the synthesis of glutathione, a tripeptide comprised of the amino acids glutamate, cysteine and glycine.

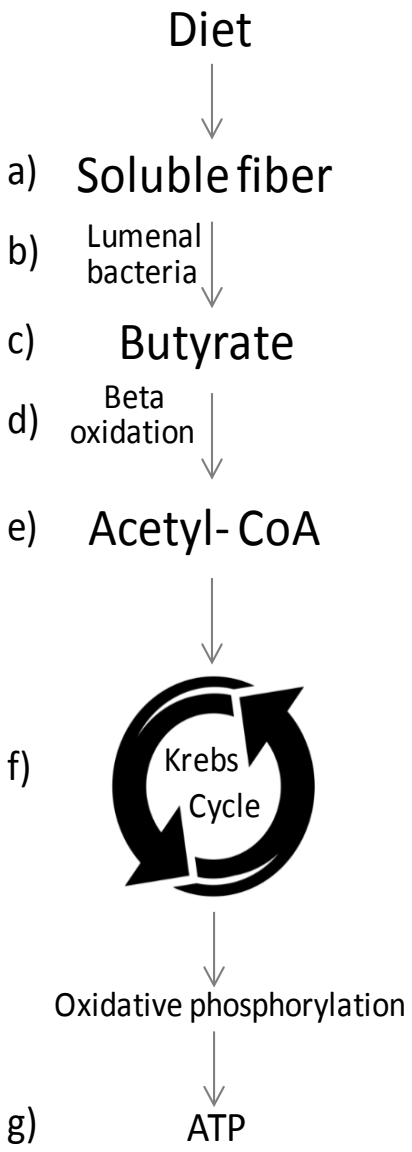

Figure 1: Normal Colonocyte bioenergetics: Soluble dietary fiber (a) is absorbed by lumenal bacteria (b) where it undergoes fermentation to short chain fatty acids (c, i.e. butyrate) that is secreted into the colonic lumen. Lumenal butyrate is then absorbed by colonocytes and transported to mitochondria where it is processed via beta-oxidation (d) to yield acetyl-CoA, which is metabolized via the Krebs cycle (f). The Krebs cycle then generates reducing equivalents (NADH and FADH2), which provide the electrons needed to power oxidative phosphorylation and the biosynthesis of ATP (g) that supplies the chemical energy to fuel nearly all energy requiring reaction in the body.

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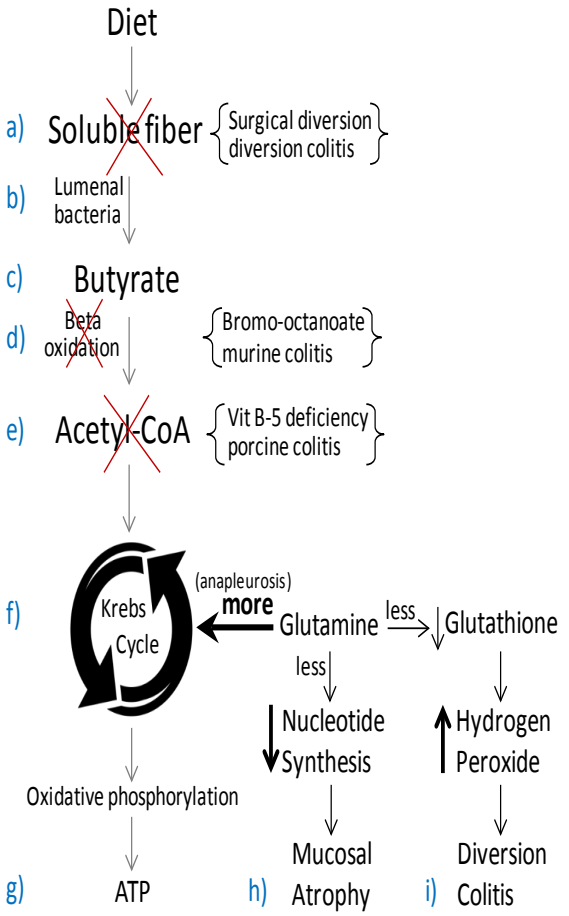

Figure 2: Mechanism of diversion colitis: The inhibition of energy flow by the absence of soluble fiber after colonic surgical by-pass (a), inhibition of beta-oxidation by 2-bromo-octanoate (b) or inhibition of Co-enzyme-A synthesis by vitamin B-5 deficiency (c) results in increased anapleurotic glutamine metabolism to replace Krebs cycle intermediary metabolites (f). Glutamine provides nitrogen for nucleotide synthesis and its redirection into the Krebs cycle (anapleurosis) for energy metabolism reduces its availability for nucleotide synthesis leading to colonic epithelial cell (mucosal) atrophy (h). Glutamine is also the precursor to glutamate, which is necessary for glutathione synthesis. "Diversion" of glutamine for anapleurotic metabolism decreases its availability for the biosynthesis of glutathione, which is critical for the elimination of cellular hydrogen peroxide. This leads to increased colonocyte hydrogen peroxide and subsequent diffusion through the cell membrane to the extracellular space resulting in colitis (i). Hydrogen peroxide increases paracellular colonic epithelial permeability by oxidizing intercellular tight junctional proteins thereby facilitating bacterial translocation into the sterile lamina propria, which stimulates a mucosal inflammatory response. Hydrogen peroxide is also a potent neutrophil chemotactic agent that attracts neutrophils into the colonic epithelium further augmenting the inflammatory response leading to colitis.

Glutathione serves as reducing co-factor for glutathione peroxidase and together they constitute the main hydrogen peroxide (H_2O_2) neutralizing anti-oxidant enzyme system in the cell. Glutamine input also contributes directly to the availability of cysteine and glycine for the biosynthesis of glutathione [11]. Thus, glutamine is a critical precursor for glutathione synthesis [13].

The utilization of additional glutamine for bioenergetic requirements imposes enhanced demands for glutamine metabolism that the cell may not be able to supply**.** A contributing factor may be the dependence of the colonic epithelium on arterial blood as a source of amino acids (including glutamate and glutamine) since there is little or no transfer of amino acids from the lumen of the large intestine [14].The dependence on glutamine as an energy source can result in the sequestration of glutamine away from glutathione biosynthesis and culminating in insufficient glutathione for the reduction (neutralization) of cellular H_2O_2, which can then accumulate within colonocytes. The critical role of glutathione for the elimination of cellular H_2O_2 can be seen in glutathione peroxidase knockout mice that lack this key enzyme needed to utilize glutathione for the neutralization (reduction) of H_2O_2. Knockout mice lacking glutathione peroxidase develop colitis [15].

Hydrogen peroxide has unique properties that promote the development of colitis. Hydrogen peroxide is cell membrane permeable and a potent oxidizing agent. It will diffuse through the colonocyte cell membrane to the extracellular space causing oxidative damage to colonic epithelial intercellular tight junctional proteins thereby increasing paracellular permeability [16-19]. The increase in epithelial paracellular permeability facilitates lumenal bacterial penetration into the sterile lamina propria, which attracts subjacent intravascular neutrophils into the colonic epithelium leading to colitis. This mechanism is supported by studies showing tata intra-rectal application of N-acetylcysteine reduces inflammation and oxidative DNA damage in the excluded colonic epithelium in experimental diversion colitis [20]. N-acetylcysteine supplies cysteine for the synthesis of glutathione implying that the oxidant responsible for inflammation and DNA damage in this model of diversion colitis is H_2O_2. Hydrogen peroxide is also a powerful neutrophilic chemotactic agent that attracts neutrophils up a concentration gradient to the source of its secretion [21]. Studies have shown that H_2O_2 secreted by injured tissue functions as a paracrine chemotactic signaling agent to recruit white blood cells to the source of tissue damage [22]. Thus, colonocyte hydrogen is poised to cause colitis if produced in excess.

Taken together, thedata suggest that colonic inflammation developing as a consequence of diversion

colitis in humans, inhibition of beta-oxidation with 2-bromo-oxtanoate in mice or vitamin B-5 deficiency in pigs (vitamin B-5 is needed for Coenzyme-A synthesis) is secondary to a common mechanism arising as a consequence of the increased anapleurotic utilization of glutamine to compensate for the bioenergetic disruption in butyrate (SCFA) metabolism. This ultimately leads to a "diversion" of cellular glutamine to satisfy cellular bioenergetic requirements at the expense of epithelial growth and glutathione biosynthesis. The combined effect of glutamine restriction on epithelial renewal and glutathione synthesis culminates in mucosal atrophy with an accompanying rise in cellular H_2O_2respectively.

H_2O_2 subsequently diffuses through the colonocyte cell membrane to the extracellular space leading to colitis. This mechanism defines thedisruption of energy flow at any site in the pathway from soluble fiber to acetyl-CoA as an oxidative stressor that leads to pathologic colonocyte accumulation of $H_2O_2$and subsequent colitis.

In summary, within the setting of fecal stream diversion, colonocyte anapleurotic metabolism of glutamine creates a relative deficiency of this amino acid leading to colonic mucosal atrophy and diminished glutathione synthesis. The decrease in colonocyte glutathione precludes efficient H_2O_2 elimination leading to a buildup of H_2O_2 within colonocytes. H_2O_2 easily diffuses through the colonic epithelial cell membrane to the extracellular micro-environment where its unique properties of long life, potent oxidizing potential and the ability to attract white blood cells (neutrophilic chemotaxis) combine to promote oxidative disintegration of colonic epithelial tight junctional proteins while attracting white blood cells into the epithelium, both of which lead to colonic inflammation and eventual diversion colitis.

5. Bioenergetics Based Therapeutics

This mechanism of disease suggests that the appropriate therapeutic goal in diversion colitis should be to restore normal colonocyte bioenergetic functionality by reducing anapleurotic metabolism of glutamine to normal levels in order to allow for increased nucleotide and glutathione biosynthesis that can be used by colonocytes to reverse mucosal atrophy and eliminate excess hydrogen peroxide respectively, which contributes to the resolution of diversion colitis. This bioenergetic model suggests a specific mechanism of action for common therapeutic agents used in diversion colitis, which is discussed below.

5.1. Soluble Fiber

Soluble fiber is the only therapeutic intervention with the potential of normalizing bioenergetics for both lumenal flora and colonic epithelium because it provides the natural energetic substrate for the bacterial microbiome while its fermentation products (i.e. butyrate) are the preferred fuel for colonocyte beta-oxidation, which provides most of the energy for the colonic epithelium (**Figure 1**).The benefit of this therapy lies in its potential to reestablish normal bioenergetics in both resident symbiotic lumenal flora and colonic epithelial cells. Based on this proposed bioenergetic model; increased energy flow through beta-oxidation decreases anapleurotic metabolism of glutamine allowing for increased glutathione synthesis and reduction of colonocyte hydrogen peroxide, which contributesto the resolution of colitis.

Studies have shown that fiber supplemented enteral nutrition increased fecal butyrate in addition to butyrate producing bacteria while topical therapy of the colorectal mucosa using a solution of fibers in patients with a colostomy is reported to reduce inflammation [23-25]. Conversely, decreased intake of dietary carbohydrate has been shown to reduce concentrations of butyrate and butyrate-producing bacteria in feces [26].

Soluble fiber may also have a beneficial effect in reversing colonic bacterial dysbiosis (alteration in diversity, stability or abundance of symbiotic bacterial flora) [27-29]. The drawback of this therapy is its reliance on bacterial flora whose inflammation associated dysbiosis may impede adequate fermentation of undigested carbohydrate to butyrate. Additionally, colonic inflammation is reported to impair the absorption and beta-oxidation of butyrate, which may impede full restoration of colonocyte bioenergetics [5].

5.2. Fecal Bacteria

Transplantation of bacteria alone is not expected to result in resolution of mucosal inflammation because it does not resolve the lack of fermentable carbohydrate in the excluded colonic segment, which is needed for the production of butyrate (and other SCFAs) that is used as

an energy source by the colonic epithelium. A synbiotic composed of fermentable carbohydrate (prebiotic) and butyrate producing bacteria (probiotic) was reported to be superior to prebiotic or probiotic alone for reduction of inflammation in ulcerative colitis and may be a consideration for diversion colitis if severe dysbiosis prevents bacterial fermentation of suitable amounts of butyrate [30]. Fecal transplantation may contain fermentable carbohydrates and SCFAs in addition to butyrate producing bacteria that can have a beneficial effect on mucosal inflammation [31].Donor fecal matter contains other organisms such as archaea, viruses, fungi and protists whose long term effect in the recipient is unknown [32].

5.3. Butyrate:

Being the natural substrate for beta-oxidation, butyrate can establish normal colonocyte bioenergetics and reduce the need for glutamine anapleurosis.This decreases colonocyte hydrogen peroxide by increasing glutathione which, under this bioenergetic model, contributes to the resolution of colitis. In support of this interpretation, studies have shown that butyrate can significantly increase glutathione levels in the large intestine [33]. In other studies, butyrate was also shown to protect against H_2O_2 induced oxidant DNA damage [34]. Topical treatment with butyrate (or SCFAs) is reported to improve inflammation in diversion colitis [35-38]. The drawback with butyrate is its inability to serve as substrate for lumenal flora and thus cannot directly contribute to normalization of existing bacterial dysbiosis. In support of this view, studies have reported no improvement in dysbiosis after treatment of diversion colitis with SCFAs [39]. Absorption and beta-oxidation of butyrate are reported to be impaired by inflammation [5].

5.4. Glutamine

Studies examining the therapeutic effect of glutamine on diversion colitis are very limited. Studies of experimental diversion colitis in a rodent model report significant improvement after glutamine enemas twice weekly beginning 4 weeks after surgery for a total of 8 weeks 1). Other studies showed that oral glutamine supplementation was effective in preventing colonic mucosal atrophy in rats subjected to loop colostomy [9]. Studies comparing the effectiveness of glutamine, psyllium, and short chain fatty acids administered as an enema in a rodent model of diversion colitis observed that glutamine prevents mucosal atrophy while psyllium prevents muscular atrophy and short chain fatty acids are most effective in decreasing severity of mucosal inflammation [12]. The effect of glutamine in prevention of mucosal atrophy is supported by studies in rodent models of colitis showing that crypt depth and colonic mucosal DNA content was significantly greater after administration of oral or rectal glutamine compared to oral or rectal SCFAs [40].

The effect of glutamine on mucosal integrity and epithelial proliferation can be explained by the contribution of glutamine to nucleotide synthesis. Glutamine derived nitrogen contributes directly to *de novo* biosynthesis of both purines and pyrimidines [11]. This would allow for resumption of normal epithelial cell division and reversal of mucosal atrophy. Finally, as expected, glutamine supplementation is reported to increase intestinal glutathione levels (41,42). Increased intestinal glutathione (critical for cellular H_2O_2neutralization) will convey additional protection against oxidative damagedue to hydrogen peroxide, which is postulated to have a causal role in colonic inflammation under this bioenergetic model of diversion colitis.

5.5. N-acetylcysteine

N-acetylcysteine (NAC) provides the amino acid cysteine for glutathione synthesis [43, 44]. Glutathione is a tri-peptide composed of glycine, cysteine and glutamate that is utilized as a reducing co-factor for glutathione peroxidase, which is responsible for the neutralization of the majority of H_2O_2 produced in the colonocyte. Experimental studies in a rodent model of diversion colitis treated with NAC enemas have demonstrated significant improvement in inflammation [45].

In other studies, NAC enemas in a rodent model of diversion colitis is reported to significantly decrease oxidative damage in colonic epithelial cells implying that hydrogen peroxide is the causal oxidizing agent being targeted by this intervention [46].

In summary, reduction of colonocyte $H_2O_2$by enhanced glutathione synthesis and availability is likely a significant contributing factor to the decrease in

mucosal inflammation and colonocyte oxidative damage subsequent to NAC enemas. The drawback of NAC is its inability to restore normal bacterial or colonocyte bioenergetics and there is no indication that NAC can directly provide nitrogen precursors for nucleotide synthesis in order to normalize mucosal atrophy. There is no evidence that NAC can correct lumenal bacteria dysbiosis.

6. Discussion

Bioenergetics concerns energy flow through living systems and describes how living organisms acquire and transform energy in order to perform biological work. Metabolic pathways are the vehicle through which energy flow take place and are essential to bioenergetics [47]. The data suggest that disruption of metabolic pathways involved in the energy flux beginning with and sustained by colonic soluble fiber initiates colonocyte metabolic adjustments that lead to mucosal inflammation and diversion colitis. The absence of lumenal soluble fiber for butyrate (SCFA) production engenders a secondary (functional) colonocyte deficiency of glutamine due to increase anapleurotic use of this amino acid as an energy source. This diverts glutamine away from nucleotide and glutathione synthesis resulting in mucosal atrophy and elevated colonocyte H_2O_2respectively. The increase in colonocyte H_2O_2 leads to mucosal inflammation after diffusion to the extracellular colonocyte microenvironment by a combined effect of oxidative disintegration of epithelial tight junctional proteins and H_2O_2 induced chemo-attraction of neutrophils into the colonic epithelium. This disease model specifies that interruption in the flow of energy from lumenal soluble fiber to acetyl-CoA leads to colitis because glutamine is "diverted" away from glutathione synthesis leading to increased colonocyte H_2O_2 and mucosal inflammation.

The data suggest that optimal therapeutic effectiveness in diversion colitis is maximized by a combination of soluble fiber, SCFAs and glutamine. Soluble fiber provides natural substrate for lumenal bacteria to help correct bacterial dysbiosis while generating SCFAs via bacterial fermentation to help normalize colonocyte bioenergetics. Soluble fiber such as psyllium can form a gel within the excluded colonic lumen and may provide a temporary bulk effect that contributes to reversing colonic smooth muscle atrophy [48].

Administered short chain fatty acids (butyrate) are absorbed by colonocytes and directly enter colonocyte metabolism thereby by-passing the need for lumenal bacterial fermentation. Once integrated into the colonocyte bioenergetic pathway (beta-oxidation), SCFAs (butyrate) can reduce the energetic demand for anapleurotic metabolism of glutamine, which increases its (glutamine's) availability for glutathione synthesis. Increased glutathione synthesis lowers cellular H_2O_2 contributing to the resolution of inflammation. Finally, glutamine provides nitrogen for nucleotide biosynthesis and contributes to the resolution of mucosal atrophy by supporting the high mitotic index (DNA replication) and turnover (cell division) rate of the colonic epithelium. Glutamine also supplies additional substrate for anapleurotic metabolism and glutathione synthesis, which provides needed energy and reducing equivalents respectively. Reducing equivalents contributed by glutathione are critical for neutralization (reduction) of colonocyte hydrogen peroxide. Oral glutamine supplementation may help reduce the frequency of enemas needed to provide topical therapy for diversion colitis.

The overlapping effect of these three therapeutic agents (soluble fiber, butyrate, glutamine) contributes to the resolution of diversion colitis by improving colonic muscular atrophy, addressing colonic bacterial dysbiosis by supplying fermentable lumenal substrate, normalizing colonocyte bioenergetics with a mass-action effect of SCFAs to "kick-start" colonocyte beta-oxidation, reversing mucosal atrophy by providing a source of nitrogen for nucleotide synthesis to support colonic epithelial cell division, and ultimately reducing inflammation by correcting impaired colonic epithelial redox homeostasis with enhanced biosynthesis of glutathione to neutralize colonocyte hydrogen peroxide. This suggests that a combination therapy of all three will provide a superior therapeutic effect compared to any one agent alone and result in contemporaneous improvement in mucosal inflammation in addition to mucosal and muscular atrophy; the three cardinal histological manifestations of diversion colitis. This combination therapy has been previously suggested based on experimental empirical evidence [12].

The endoscopic and histologic similarities between diversion and ulcerative colitis have been previously

noted [49-51]. The histologic resemblance of diversion colitis to ulcerative colitis has prompted authors to suggest a pathogenetic link between both inflammatory disorders [52]. Case reports of diversion colitis in which the clinical and histological features are indistinguishable from ulcerative colitis support a common pathogenetic element [53]. Both conditions are characterized by epithelial dysfunction and are reported to respond to the topical application of SCFAs [34, 49-50].

Studies report significantly decreased colonic SCFAs in individuals with ulcerative colitis in relapse while diversion colitis is defined by the total absence of soluble fiber generated SCFAs [54]. Diversion colitis is 3x more common in the setting of pre-existing ulcerative colitis and, analogous to the distal preponderance of disease in ulcerative colitis, diversion colitis shows more pronounced inflammatory changes in the distal sections of diverted bowel [55-56]. These similarities suggest common elements in the pathogenesis of both conditions.

When integrating a bioenergetic disease model of diversion colitis with data reporting impaired colonic beta-oxidation and significantly increased non-inflamed colonic mucosal H_2O_2 in individuals with ulcerative colitis, an inclusive mechanism of disease emerges suggesting that diversion colitis is initiated as a result of exposure to a single defined oxidative stressor (absence of soluble fiber) whereas the development of ulcerative colitis follows exposure to the cumulative effect of more than one environmental oxidative stressor, some of which interrupt colonocyte bioenergetics (i.e. decreased lumenal SCFAs) while others increase colonocyte hydrogen peroxide by other mechanisms (i.e. stress) (an oxidative stressor is a trigger that increases colonocyte H_2O_2) [57-59]. Based on this bioenergetic model, the experimental colitides cause by inhibition of beta-oxidation in rats and vitamin deficiency colitis in pigs (described above) are examples of defined oxidative stressors leading to disrupted bioenergetics with a subsequent buildup of colonocyte H_2O_2followed bythe development of colitis.

Stated differently, diversion colitis is iatrogenically initiated with a single defined oxidative stressor resulting from surgical diversion of the fecal stream leading to the absence of soluble fiber and disrupted colonocyte bioenergetics. Butyrate provides about 70% of colonocyte energy and the colonic epithelium makes up for this significant energy deficit by channeling glutamine into the Krebs cycle (anapleurotic metabolism). This diverts glutamine away from nucleotide synthesis leading to mucosal atrophy. Anapleurotic metabolism of glutamine also redistributes this amino acid away from glutathione synthesis leading to increased colonocyte H_2O_2 and colitis.

In contrast, ulcerative colitis is characterized by exposure to multiple contemporaneous environmental oxidative stressors leading to the buildup of colonocyte H_2O_2 and the development of mucosal inflammation. Studies have reported significantly elevated levels of H_2O_2 in the non-inflamed colonic mucosa of individuals with ulcerative colitis implying that the buildup ofH_2O_2 anteceded the development of colitis and suggesting a causal role for H_2O_2 in the pathogenesis of this illness [58]. This is supported by clinical results obtained by the targeted therapeutic reduction of colonic hydrogen peroxide, which resulted in histologic remission (complete mucosal healing) in 85% of 36 individuals with refractory ulcerative colitis [60].

In summary, we are all exposed to environmental oxidative stressors but a subset of individuals are “environmentally selected” to develop ulcerative colitis upon exposure to environmental oxidative stressors because of a predisposing genetic makeup encoding for a diminished reductive capacity that facilitates the buildup of H_2O_2. This view is supported by studies showing that the concentration of red blood cell glutathione is a heritable trait with additional studies of healthy individuals reporting a wide interindividual range of plasma and intracellular glutathione concentrations of approximately one order of magnitude [61-63]. This places a portion of the population in the low range of reductive (anti-oxidant) capacity and genetically susceptible to H_2O_2 induced buildup and oxidant injury. By comparison, there is no oxidative environmental selection process operative in diversion colitis because surgical diversion is performed for reasons unrelated to individual reductive (anti-oxidant) capacity, which is expected to be normal in most individuals prior to surgical diversion. Thus, the very high incidence of diversion colitis suggests that colonic diversion is a potent oxidative stressor that may benefit from prophylactic oral glutamine therapy prior to undergoing these surgical procedures to prevent

colonic glutamine deficiency. A role for colonocyte H_2O_2 in the pathogenesis of diversion colitis can be tested by measuring colonic epithelial H_2O_2. A method for measuring H_2O_2in colonic mucosal biopsy samples has been previously reported [56].

7. Conclusion

A bioenergetic analysis of diversion colitis suggests a more complex metabolic derangement caused by the absence of lumenal short chain fatty acids than previously thought. The data suggest that increased utilization of glutamine as an energy substrate diverts this amino acid from nucleotide and glutathione biosynthesis leading to mucosal atrophy and a buildup of colonocyte hydrogen peroxide respectively. Hydrogen peroxide's unique properties of cell membrane permeability, long life, potent oxidizing potential and the ability to attract white blood cells combine to promote oxidative disintegration of colonic epithelial tight junctional proteins while attracting white blood cells into the colonic epithelium, both of which lead to colonic inflammation and eventual diversion colitis. The proposed bioenergetic model provides an evidence-based pathogenesis leading to the development of diversion colitis and a mechanism underlying the beneficial effects of common therapeutic agents used to treat this condition. It also provides a scientific basis for the treatment of diversion colitis by combining different and complementing therapeutic modalities to achieve maximum beneficial effect on inflammation, mucosal and muscular atrophy; the three characteristic histopathological manifestations of diversion colitis.

References

1. Pacheco RG, Esposito CC, Müller LC, Castelo-Branco MT, Quintella LP, Chagas VL et al. Use of butyrate or glutamine in enema solution reduces inflammation and fibrosis in experimental diversion colitis. World Journal of Gastroenterology 2012; 18: 4278-87.

2. Tominaga K., Kamimura K, Takahashi K, Yokoyama J, Yamagiwa S, Terai S et al. Diversion colitis and pouchitis: A mini-review. World Journal of Gastroenterology 2018; 24: 1734-47.

3. Leonel AJ, Alvarez-Leite JI. Butyrate: implications for intestinal function. Current Opinion in Clinical Nutrition & Metabolic Care. 2012; 15: 474-9.

4. Houten SM, Wanders RJA. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. Journal of iInherited Metabolic disease. 2010; 33:469-77.

5. De Preter V, Arijs I, Windey K, Vanhove W, Vermeire S, Schuit F et al. Impaired butyrate oxidation in ulcerative colitis is due to decreased butyrate uptake and a defect in the oxidation pathway. Inflammatory Bowel Diseases. 2012; 18: 1127-36.

6. Roediger WE, Nance S. Metabolic induction of experimental ulcerative colitis by inhibition of fatty acid oxidation. British Journal of Experimental Pathology. 1986; 67: 773-82.

7. Nelson RA. Intestinal Transport, Coenzyme A, and Colitis in Pantothenic Acid Deficiency. The American Journal of Clinical Nutrition. 1968; 21: 495-501.

8. Cherbuy C, Darcy-Vrillon B, Morel MT, Pégorier JP, Duée PH et al. Effect of germfree state on the capacities of isolated rat colonocytes to metabolize n-butyrate, glucose, and glutamine. Gastroenterology. 1995; 109: 1890-9.

9. Paulo FL. Effects of oral supplement of L-glutamine on diverted colon wall. Journal of cellular and molecular medicine. 2002; 6: 377-82.

10. Yang C, Ko B, Hensley CT, Jiang L, Wasti AT, Kim J et al. Glutamine oxidation maintains the TCA cycle and cell survival during impaired mitochondrial pyruvate transport. Molecular cell. 2014; 56: 414-24.

11. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nature Reviews Cancer. 2016; 16: 619-34.

12. Pathak M, Srinivas M, Shariff A. Prevention of Histological Changes after Colonic Diversion in Rats: An Experimental Study. Journal of Neonatal Surgery. 2017; 6: 26.

13. Amores-Sánchez MI, Medina MÁ. Glutamine, as a precursor of glutathione, and oxidative stress. Molecular Genetics and Metabolism. 1999; 67: 100-5.

14. Blachier F, Boutry C, Bos C, Tomé D. Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines. The American journal of clinical nutrition. 2009; 90: 814S-21S.

15. Esworthy RS, Aranda R, Martín MG. Mice with combined disruption of Gpx1 and Gpx2 genes have colitis. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2001; 281: G848-G855.

16. Gangwar R, Meena AS, Shukla PK, Nagaraja AS, Dorniak

PL, Pallikuth S et al. Calcium-mediated oxidative stress: a common mechanism in tight junction disruption by different types of cellular stress. Biochemical Journal. 2017; 474: 731-49.

17. Rao RK, Li L, Baker RD, Baker SS, Gupta A et al. Glutathione oxidation and PTPase inhibition by hydrogen peroxide in Caco-2 cell monolayer. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2000; 279: G332-40.

18. Parrish AR, Catania JM, Orozco J, Gandolfi AJ et al. Chemically induced oxidative stress disrupts e-cadherin/ catenin cell adhesion complex Toxicol Sci. 1999; 51: 80-86.

19. Rao RK, Baker, RD, Baker SS, Gupta A, Holycross M et al. Oxidant induced disruption of intestinal epithelial barrier function: role of protein tyrosine phosphorylation. Gastrointest Liver Physiol. 1997; 36: G812-G823.

20. Martinez CAR, Ribeiro ML, Gambero, A et al. The importance of oxygen free radicals in the etiopathogenesis of diversion colitis in rats. ActaCirurgicaBrasileira. 2010; 25: 387-95.

21. Klyubin IV, Ksenia MK, Gamaley IA. Hydrogen peroxide induced chemotaxis of mouse peritoneal neutrophils. Eur J Cell Biol. 1996; 70: 347-51.

22. Niethammer P, Grabher C, Look AT. A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. Nature. 2009; 459: 996-9.

23. Majid HA, Emery PW, WhelanK. Faecalmicrobiota and short-chain fatty acids in patients receiving enteral nutrition with standard or fructo-oligosaccharides andbre-enriched formulas. J Hum Nutr Diet. 2011; 24: 260-8.

24. O'Keefe SJ, Ou J, Delany JP. Effect of fiber supplementation on the microbiota in critically ill patients. World J GastrointestPathophysiol. 2011; 2: 138-45.

25. de Oliveira-Neto JP, de Aguilar-Nascimento JE. Intraluminal irrigation with fibers improves mucosal inflammation and atrophy in diversion colitis. Nutrition. 2004; 20: 197-199.

26. Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley GE et al. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. Appl Environ Microbiol. 2007: 73; 1073-8.

27. Alonso VR, Guarner F. Linking the gut microbiota to human health. British Journal of Nutrition. 2013; 109; S2: S21-S26.

28. Valcheva R, Koleva P, Martinez I, Walter J, Gänzle MG, Dieleman LA et al. Inulin-type fructans improve active ulcerative colitis associated with microbiota changes and increased short-chain fatty acids levels. Gut microbes. 2018; 10: 334-57.

29. Healey G, Murphy R, Butts C, Brough L, Whelan K, Coad J et al. Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: a randomised, double-blind, placebo-controlled, cross-over, human intervention study. British Journal of Nutrition. 2018; 119: 176-89.

30. Fujimori S, Gudis K, Mitsui A, Seo T, Yonezawa M, Tanaka S et al. A Randomized controlled trial on the efficacy of synbiotic versus probiotic or prebiotic treatment to improve the quality of life in patients with ulcerative colitis. Nutrition. 2009; 25: 520-5.

31. Gundling F, Tiller M, Agha A, Schepp W, Iesalnieks I et al. Successful autologous fecal transplantation for chronic diversion colitis. Tech Coloproctol. 2015; 19: 51-2.

Bojanova DP, Bordenstein SR. Fecal transplants: what is being transferred? PLoS biology 2016; 14: e1002503.

32. Hamer HM, Jonkers DM, Bast A, Vanhoutvin SA, Fischer MA, Kodde A et al. Butyrate modulates oxidative stress in the colonic mucosa of healthy humans. Clinical Nutrition. 2009; 28: 88-93.

33. Rosignoli P, Fabiani R, De Bartolomeo A, Spinozzi F, Agea E, Pelli MA, Morozzi G et al. Protective activity of butyrate on hydrogen peroxide-induced DNA damage in isolated human colonocytes and HT29 tumour cells. Carcinogenesis. 2001; 22: 1675-80.

34. Harig JM, Soergel KH, Komorowski RA. Treatment of diversion colitis with short-chain-fatty acid irrigation. N Engl J Med. 1989; 320: 23-8.

35. Kiely EM, Ajayi NA, Wheeler RA, Malone M. Diversion procto-colitis: response to treatment with short-chain fatty acids. Journal of Pediatric Surgery. 2001; 36: 1514-7.

36. Pal K, Tinalal S, Al Buainain H, et al. Diversion proctocolitis and response to treatment with short-chain fatty acids--a clinicopathological study in children Indian J Gastroenterol. 2015; 34: 292-9.

37. Luceri C, Femia AP, Fazi M, Di Martino C, Zolfanelli F, Dolara P et al. Effect of butyrate enemas on gene expression profiles and endoscopic/histopathological scores of diverted colorectal mucosa: A randomized trial. Dig Liver Dis. 2016; 48:

27-33.

38. Neut C, Guillemot F, Gower-Rousseau C, Biron N, Cortot A, Colombel JF et al. Treatment of diversion colitis with short-chain fatty acids. Bacteriologicalstudy. GastroenterologieClinique et Biologique. 1995; 19: 871-5.

39. Kaya E, Ceylan A, Kara N, Güven H, Yildiz L et al. The effect of L-glutamine on mucosal healing in experimental colitis is superior to short-chain fatty acids. Turk J Gastroenterol. 2017; 18; 89-94.

40. Cao Y, Feng Z, Hoos A, et al. Glutamine enhances gut glutathione production. Journal of Parenteral and Enteral Nutrition. 1998; 22: 224-7.

41. Krauseselt AI, Fürst P. Glutamine (GLN) supplementation promotes glutathione (GSH) synthesis and prevents intestinal ischemia/reperfusion (I/R) injury. Clin Nutrition. 2001; 20: 59-60.

42. Rushworth GF, Megson IL. Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits. Pharmacology & Therapeutics. 2014; 141: 150-9.

43. Atkuri KR., Mantovani JJ, Herzenberg LA. N-Acetylcysteine—a safe antidote for cysteine/glutathione deficiency. Current Opinion in Pharmacology. 2007; 7: 355-9.

44. Almeida MGD, Pereira JA, Silva CMGD. Evaluation of topical n-acetylcysteine in diversion colitis. Journal of Coloproctology 2012; 32: 223-31.

45. Martinez CAR, de Almeida M G, da Silva CMG. Enemas with N-acetylcysteine can reduce the level of oxidative damage in cells of the colonic mucosa diverted from the faecal stream. Digestive Diseases and Sciences. 2013; 58: 3452-9.

46. Nelson, David L., Cox, Michael M. Lehninger: Principles of Biochemistry. New York: W.H. Freeman and Company 6thed. 2013; 24-27.

47. Fischer MH, Yu N, Gray GR, Ralph J, Anderson L, Marlett JA et al. The gel-forming polysaccharide of psyllium husk (PlantagoovataForsk). Carbohydrate Research. 2004; 339: 2009-17.

48. Steinhart AH, Brzezinski A, Baker JP. Treatment of refractory ulcerative proctosigmoiditis with butyrate enemas. American Journal of Gastroenterology. 1994; 89: 179-83.

49. Scheppach W, Sommer H, Kirchner T, Paganelli GM, Bartram P, Christl S et al. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology. 1992; 103: 51-6.

50. Komorowski RA. Histologic spectrum of diversion colitis. The American journal of surgical pathology. 1990; 14: 548-54.

Geraghty JM, Talbot IC. Diversion colitis: histological features in the colon and rectum after defunctioning colostomy. Gut. 1991; 32: 1020-3.

51. Kakizawa N, Tsujinaka S, Miyakura Y, et al. The surgical treatment of acute and severe diversion colitis mimicking ulcerative colitis: a case report. Surgical Case Reports. 2018; 4: 86.

52. Vertzoni M, Goumas K, Söderlind E, et al. Characterization of the ascending colon fluids in ulcerative colitis. Pharmaceutical Research. 2010; 27: 1620-6.

53. Thorsen AJ. Noninfectious colitides: collagenous colitis, lymphocytic colitis, diversion colitis, and chemically induced colitis. Clinics in Colon and Rectal Surgery. 2007; 20: 47-57.

54. Kleinwort A, Döring P, Hackbarth C, Heidecke CD, Schulze T et al. Deviation of the Fecal Stream in Colonic Bowel Segments Results in Increased Numbers of Isolated Lymphoid Follicles in the Submucosal Compartment in a Novel Murine Model of Diversion Colitis. Biomed Res Int. 2017; 2017: 5265969.

55. Chapman MA, Grahn, MF, Boyle MA, Hutton M, Rogers J, Williams NS et al. Butyrate oxidation is impaired in the colonic mucosa of sufferers of quiescent ulcerative colitis. Gut 1994; 35: 73-6.

56. Santhanam S, Venkatraman A, Ramakrishna BS. Impairment of mitochondrial acetoacetyl CoA thiolase activity in the colonic mucosa of patients with ulcerative colitis. GUT. 2007; 56: 1543-9.

57. Pravda J. Radical induction theory of ulcerative colitis. World Journal of Gastroenterology 2005; 11; 2371-84.

58. Pravda J, Weickert M, Wrubble L. Novel Combination Therapy Induced Histological Remission in Patients with Refractory Ulcerative Colitis. J Inflam Bowel Dis Disor. 2019; 4: 130.

59. Van'tErve TJ, Wagner BA, Ryckman KK, Raife TJ, Buettner GR et al. The concentration of glutathione in human erythrocytes is a heritable trait. Free RadicBiol Med. 2013; 65: 742-9.

60. Michelet F, Gueguen R, Leroy P, Wellman M, Nicolas A, Siest G et al. Blood and plasma glutathione measured in healthy

subjects by HPLC: relation to sex, aging, biological variables, and life habits. Clinical Chemistry. 1995; 41: 1509-17.

61. Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med. 2009; 30: 1-12.