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THE ROLE OF BUTYRIC ACID AS A PROTECTIVE AGENT AGAINST INFLAMMATORY BOWEL DISEASES

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SUMMARY

Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are pathologies characterized by a chronic inflammation of the gastrointestinal tract. Their etiopathogenesis is not yet fully understood. Immune system and heat shock proteins (Hsps) dysfunctions are considered to be among the most likely causes of these diseases. Butyrate is a short-chain fatty acid mainly produced by intestinal microflora. It has a trophic, beneficial and protective role in the colonic mucosa, and it also induces changes in Hsp levels and localization. It may therefore be a valuable complementary therapeutic agent when used alongside traditional drugs (mesalazine and corticosteroids) to treat such conditions. The administration of specific probiotic formulations in order to increase the production of butyrate in the endoluminal environment may promote clinical remission in IBD patients. Due to these characteristics, there has been keen interest in the use of butyrate as a novel therapeutic supplement in the recent years. The current findings need to be validated through further clinical trials to better define the biomolecular dynamics of butyrate in the colonocytes of IBD patients.

Introduction

Inflammatory bowel diseases (IBD) are pathologies characterized by a chronic inflammation of the gastrointestinal tract that present with abdominal pain and severe watery and bloody diarrhea. The most common inflammatory bowel diseases are Crohn's disease (CD) and ulcerative colitis (UC).

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IBDs are characterized by an early onset and extraintestinal manifestations, such as rheumatoid arthritis (1, 2). CD can affect any part of the gastrointestinal tract, on the contrary of UC, which is usually confined to the colon and rectum. The incidence of IBD in Europe and North America is 20/100000 inhabitants, indicating a link between such pathologies and the social and economic development in these countries. In fact, IBD first emerged in Northern Europe and North America, followed by the rest of Europe, South America and Japan (3). The exact etiopathogenesis of IBD is still unclear, but the most widely acknowledged mechanism involves an inappropriate interaction between the immune system of genetically susceptible individuals, the microbiota, and environmental factors (4). The standard treatment of IBD is currently based on the administration of anti-inflammatory drugs, such as mesalazine and corticosteroids, but the treatment response rates are suboptimal. Since IBD presents as a distortion of the relationship between the intestinal microbiota and the host response, probiotics and prebiotics have been proposed as possible therapeutic agents, since the balance of the intestinal microbiota can also be enhanced with a diet of non-digestible substances that can selectively promote the growth and/or the activity of one or more bacteria already present in the gastrointestinal tract or taken with probiotics. The probiotics that have been tested for the treatment of IBD include mainly lactic acid bacteria, such as different *Lactobacilli* and *bifidobacteria* strains. It has been observed that the administration of *Lactobacilli* helps to improve the inflammatory process in IBD (5). About 1–2% of colorectal cancer cases have a pathological background characterized by a chronic inflammation of the intestinal mucosa that may evolve to a minor (low-grade) or a more severe (high-grade) dysplasia, which may in turn give rise to a carcinoma in situ and finally to an invasive carcinoma through neoplastic transformation. Short-chain fatty acids, in particular butyrate, derived from the bacterial fermentation of indigestible carbohydrates, provide nutrients and growth signals for the intestinal epithelium. Butyrate has been shown to prevent apoptosis and the subsequent mucosal atrophy in normal colonocytes (6).

The intestinal mucosa

The mucosa surrounding the intestinal lumen acts as a physical barrier to the bacterial species living in the intestine (microbiome), and consists of a single layer of epithelial cells. An intact mucosal barrier is essential for intestinal wellbeing, and rapid healing of this layer following injury is crucial for disease prevention. The epithelial barrier stops any potentially harmful substances from passing into the lumen. It also regulates the flow of nutrients, solutes, and ions into the underlying mucosa. Molecules can cross the epithelium through the apical plasma membrane via specialized channels (transcellular pathway). They can also pass across the paracellular space between epithelial cells through the pores created by junction proteins (paracellular pathway) (7). The mucosal cells are tied to each other by different intercellular junctions: *Zonula Occludens* (tight junctions (TJs)) and *Zonula Adherens* (adherens junctions), collectively known as the apical junction complex (AJC), gap junctions and desmosomes. The AJC regulates cell polarity and enables the selective permeability barrier function. Coordination of the TJ proteins with the actin cytoskeleton is required to enable the processes of endocytosis and intracellular signaling pathways. In addition, commensal bacteria are involved in the maintenance of host barrier homeostasis by modulating cell renewal, facilitating wound healing, and reorganizing the TJs (8). The selective permeability of the barrier is determined by claudin proteins and the expression patterns of charged amino acids in their extracellular loops which generate different sized pores through which solute transfer occurs. TJ stability and dynamic formation to accommodate intestinal epithelial cell (IEC) turnover, that occurs every four to five days, are essential to maintain the integrity of the mucosal barrier. Thus, TJ proteins are continuously internalized via clathrin-mediated endocytosis. TJ turnover and claudin expression can be modified by cytokines released by leucocytes migrating across epithelial barriers. *In vitro* experiments have shown that TNF-α downregulates claudin proteins, increasing the permeability of the intestinal mucosa. TJ recycling can also be altered by inflammation-inducing pathogenic bacteria, causing increased claudin internalization and enhanced permeability (9).

The physiological regulation of barrier homeostasis relies on tightly controlled signal transduction pathways that converge on the cytoplasmic TJ proteins.

The microbiota

The intestine of the mammalian fetus is sterile while *in utero*. At birth, the intestinal microbiota is acquired by ingesting maternal anal or vaginal organisms (10). The effect of the first inoculum may be lifelong, regulating the development of the immune system and the intestinal microbiota. The bacterial composition of the inoculum received at birth and the structure of the host's intestinal epithelium, as well as diet, can affect the density and composition of the microbiota. The intestinal microbiota plays a critical role both in healthy individuals and progression of diseases such as inflammatory bowel disease and colon cancer. Each individual's intestine is colonized by up to 10^{14} bacterial cells of at least 160 different species, that sums up to a population about 10 times larger than that of the somatic and germ cells harbored by the human body (11). The microbiota exerts diverse physiological functions such as inhibition of pathogenic bacteria and synthesis of short-chain fatty acids; stimulation of nutrient absorption, intestinal immune system and cellular renewal; synthesis of vitamins and amino acids; and the decomposition of protein compounds (12). A relationship has been discovered between the microbiota and diseases such as obesity and IBD (13). Changes in the microbiota composition are mainly influenced by diet (14) and age, as well as genetic factors (15). Low molecular weight metabolites produced by the intestinal microbiota play a direct role in the maintenance of intestinal health and in the onset of disease. Microorganisms and associated genomes are likely to produce compounds that directly influence host life processes. The beneficial effects of lactic acid-producing organisms in fermented milk products were first discovered in the beginning of the 20th century by Metchnikoff (16). Fermented milk products contain natural compounds and microorganisms, known as probiotics, that are thought to be beneficial to human health. The use of probiotics to help maintain intestinal homeostasis and ameliorate specific gastrointestinal pathologies has generated enormous interest in recent years. Some strains of probiotic microbes can reduce intestinal permeability through direct effects on the IECs and reduce inflammation. IECs have signaling mechanisms that activate the immune system following various stimuli. Nuclear factor-kappaB (Nf-κB) is a transcription factor present in the cytoplasm in an inactive form, bound to inhibitory molecules of the IκB family (17). In response to proinflammatory stimuli, IκB becomes phosphorylated and detaches from Nf-κB, allowing it to migrate from the cytoplasm to the nucleus and begin the activation of the transcription of specific genes (18). *Lactobacillus plantarum* has been shown to inhibit the degradation of IκB and, consequently, the activity of NF-κB *in vitro* (19). Another molecular target modulated by probiotics is PPARγ, a nuclear receptor that can regulate the level of intestinal inflammation by inhibiting the activity of NF-κB; PPARγ has been found in small amounts in the IECs of patients with IBD (20). Probiotics can implement their beneficial activity through several mechanisms (Figure 1).

The short-chain fatty acids

The short-chain fatty acids (SCFA) are small fatty acids, containing one to six carbon atoms, with an anionic behavior. They derive from the bacterial fermentation of polysaccharide, oligosaccharide, and glycoprotein precursors in the colon (21). The fermentation process involves many reactions and metabolic processes leading to anaerobic microbial breakdown of organic matter that produces energy for microbial growth and other metabolic end products for host use. The production rates of SCFAs vary in the diverse tracts of the intestine, depending on the bacterial species present in the microbiome and the host diet (that is responsible for the availability of the necessary precursors for the production of SCFAs). Bacterial fermentation and proliferation are highest in the proximal colon where substrate availability is greatest (22). Since specific species such as *Bifidobacterium* and *Lactobacillus* have been associated with health benefits, it would be useful to identify specific bacteria with defined metabolic functions. SCFAs are rapidly absorbed by the caecum and colon, and have been shown to enhance

sodium absorption and bicarbonate secretion. The absorption of SCFAs can take place through two mechanisms: diffusion of protonated SCFAs and anion exchange. The main SCFAs, acetate, propionate and butyrate, are absorbed at comparable rates in the different regions of the colon. SCFAs have several roles: they provide nutrients for the colonic epithelium, as well as acting as modulators of colonic and intracellular pH, and regulators of proliferation, differentiation and gene expression (23). SCFAs are the main energy source of colonocytes, and they are also fundamental for mucous membrane growth control. SCFAs regulate the proliferation of colonic epithelial cells, by not only favoring the re-epithelialization of the normal mucous membrane, but also by preventing the proliferation of tumor cells by inhibiting DNA synthesis and re-establishing natural apoptosis. SCFAs in general, and, in particular, butyrate, enhance the growth of bacteria such as *Lactobacilli* and *Bifidobacteria*, that play a very important role in the physiology of colonocytes (24).

Butyric acid: production and metabolism

Butyric acid is a SCFA with four carbon atoms that causes the characteristic smell of rancid butter. It is also known as butanoic acid or butyrate, and its formula is $CH_3CH_2CH_2$-COOH. Butyrate is produced through a fermentation process involving bacteria feeding on prebiotic products in anaerobic conditions. The dietary fiber is fermented by microbes into butyrate in the lumen of the colon, and transported into the colonocytes. The absorption of butyric acid by colonocytes occurs through various mechanisms that change depending on the location. On the apical side, the absorption takes place through passive diffusion and the use of non-ionic transporters like monocarboxylate transporter (MCT) type 1 and sodium-coupled monocarboxylate cotransporter (SMCT). On the basolateral membrane, there is a carrier-mediated HCO_3 gradient-dependent anion–butyrate exchange system. The transport of butyrate appears to be influenced by the pH levels of the intestinal lumen. Butyric acid absorption uses a pH-activated, electroneutral anion exchange system that functions best at a pH value of 5,5 (25). MCTs cou-

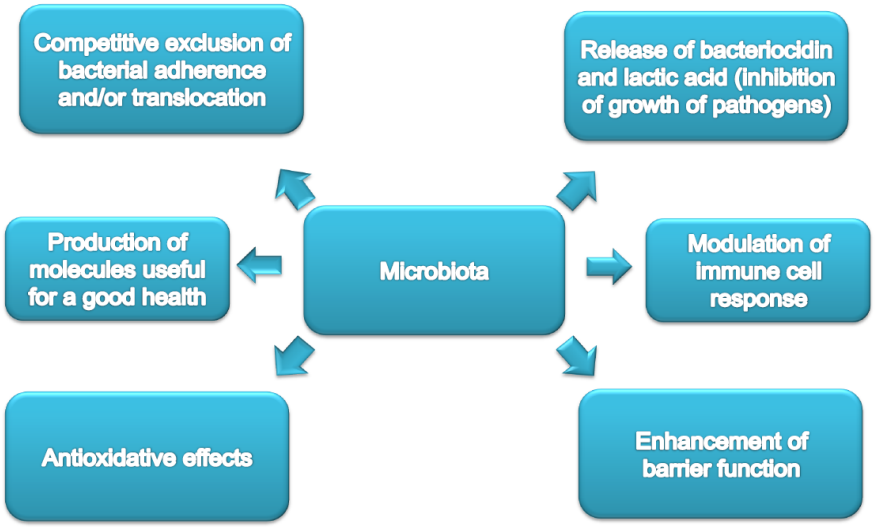

Figure 1: Microbiota has many roles in the regulation of host processes. It is able to change the response of immune cells through mechanisms involving (for example) the release of immune-regulatory molecules. It changes the intestinal environment through the release of compounds that may modify pH (such as lactic acid) and bacteriocidin to inhibit the growth of pathogens. It interacts with colonocytes inducing in them mechanisms to increase tight junctions (higher permeability). Moreover, it produces compounds important for the health of the subject (for example SCFA) and molecules with anti-oxidative effects. It competes with the other bacterial species inhabiting the intestine for nutritive substances, adherence and translocation.

pled with H+ ions are easily saturated and can be inhibited by compounds such as acetate, propionate, lactate and pyruvate. SMCTs function with sodium ion, nicotinate and ketone bodies. This family of receptors includes SMCT1 and SMCT2. Studies on colon cancer cells have shown that these two transporters may play a role in tumorigenesis. In fact, in colon cancer cells, their expression is silenced and the inhibitory effect of butyrate is prevented, confirming the theory that these two genes act as tumor suppressors (26). The relationship between IBD and colon carcinoma resides in the fact that chronic inflammatory diseases (such as IBD) increase the risk of developing tumors. Butyrate is used by colonocytes primarily as an energy source to complete oxidation which yields CO_2 through the fatty acid oxidation pathway. The microbiome regulates host metabolism, having a strong effect on the energy homeostasis of the colon. In fact, metagenomic sequence data show that the intestinal microbiome is highly enriched in genes involved in energy production and metabolism. From the results of a comparative study on colonocytes derived from germfree (GF) and conventional (CONV-R) mice (with normal but undefined microbiota), it has emerged that colonocytes from GF mice subsist in an energy-deprived state and show a decreased expression of enzymes that catalyze key reactions of the tricarboxylic acid (TCA) cycle. This leads to a marked decrease in the NADH/NAD+ ratio, oxidative phosphorylation, and ATP levels. An analysis of the transcriptome of the colonocytes from GF and CONV-R mice identified 624 upregulated genes and 813 downregulated genes in GF compared to CONV-R. A proteomic comparative analysis with two-dimensional shotgun mass spectrometry approach did not find significant differences in the levels of proteins between the GF and CONV-R samples. Data analysis using the Ingenuity Pathway Analysis (IPA) and Kegg (Kyoto Encyclopedia of Genes and Genomes) identified genes related to butyrate metabolism. Genes linked to β-oxidation and the TCA cycle are downregulated in GF colonocytes, confirming that bacteria exert a positive effect on the regulation of these processes. Consequently, there is a marked decrease in NADH/NAD+, oxidative phosphorylation, and ATP levels. These alterations eventually lead to the death of colonocytes through the autophagy pathway (27).

Bacterial producers of butyric acid

The majority of butyrate-producing bacteria found in human faeces are highly oxygen-sensitive anaerobes belonging to the Clostridial clusters IV and XIVa (28). Decreased quantities of these bacteria species have been reported in the gut of IBD patients (29, 30), thus making them potential probiotic candidates for the treatment of IBD. Recently, considerable progress has been made in the isolation of these strictly anaerobic butyric acid-producing bacteria from the human intestine. It has been shown that lactic acid, produced *in vitro* by lactic acid bacteria, is used by some Clostridial cluster XIVa species for the production of high concentrations of butyric acid (28). This mechanism is called cross-feeding and it could explain why administration of lactic acid bacteria to IBD patients can, in certain cases, be beneficial due to the stimulation of butyric acid production. The ideal probiotic would therefore be a colonizing bacterium that combines systemic anti-inflammatory and immunoregulatory effects with delivery of high butyrate levels at the site of action, and that can be ingested in a stable form, such as spores. One potential problem is that bowel conditions in IBD patients may not be optimal to support the growth of these bacterial species. However, butyrate producers present in the colon are phylogenetically diverse and it has not been investigated in detail to date which specific species are decreased in IBD. Therefore, it is likely that strains with good colonizing properties can be identified. Furthermore, a symbiotic approach, using a prebiotic in combination with a butyrate producer, may help to reestablish such bacteria in individuals affected by IBD. Strains of Clostridial clusters IV and XIVa have been found to be highly successful in decreasing inflammation and necrosis in rodent IBD models. Indeed, *Faecalibacterium prausnitzii*, a species belonging to cluster IV, has been shown to be much less prevalent in the intestinal microbiota of IBD patients. This species showed great promise in counterbalancing dysbiosis in a mouse IBD model (31).

The effects of butyric acid on the health of colonocytes

It has been observed that patients with UC appear to have impaired butyrate metabolism. Butyrate enemas have been demonstrated to ameliorate inflammation in UC (32). Butyric acid, produced within the intestinal lumen by bacterial fermentation of dietary carbohydrates, has a wide variety of effects on intestinal functions. Firstly, butyric acid is the preferred source of energy of colonocytes. It also has various effects on colonocyte proliferation, differentiation and apoptosis, in addition to anti-inflammatory effects. Butyric acid can influence gene expression, since has been reported to interact with histone deacetylase (HDAC) and inhibit it. This results in hyperacetylation of histones with a consequent suppression of Nf-κB activation. Butyric acid reinforces the colonic defense barrier by increasing production of mucins and antimicrobial peptides. Moreover, it has been shown that butyric acid decreases intestinal epithelial permeability by increasing the expression of tight junction proteins. Anti-inflammatory actions, combined with a strengthening effect on the mucosal barrier integrity, are ideal properties for therapeutic compounds against IBD-like syndromes. Indeed, it has been shown that butyrate can yield positive results in the treatment of active UC (33). Therefore, butyric acid has a potential therapeutic effect against IBD, but the actual delivery into the gut is still problematic. Several mechanisms are possible. Fermentation of dietary fiber leads to butyrate production. An increase in butyrate production may result from a direct stimulation of butyrate producers or indirect actions such as metabolic cross-feeding of fermentation products from other bacterial groups (34). Butyrate has been shown to protect Caco-2 cell monolayers from *Campylobacter jejuni* invasion and translocation in a concentration-dependent manner by increasing the transepithelial electrical resistance (TEER), implying a decrease in the permeability of intestinal mucosa (35). The effects of butyrate have been investigated in a study of intestinal biopsy specimens, lamina propria mononuclear cells (LPMC) and peripheral blood mononuclear cells (PBMC) derived from 17 patients affected by CD. Butyrate treatment was found to induce a decrease in TNF production and proinflammatory cytokine mRNA expression in intestinal biopsies and LPMC of CD patients. PBMC were cultured in the presence or absence of lipopolysaccharide (LPS) and the effects of treatment with butyric acid were evaluated. The results showed a decreased expression of proinflammatory cytokines in PBMC and transmigration of NF-κB from the cytoplasm to the nucleus, while IκBα levels remained stable (36). CD and UC epithelia have been shown to be subjected to metabolic stress, leading to a decreased TEER and an increased number of pseudopodia, that is consistent with the results showing increased internalization and translocation of bacteria across mucosa (especially *E. coli*). Butyrate is able to significantly reduce the bacterial translocation across epithelia treated with Dinitrophenol (DNP), but does not ameliorate TEER in monolayers exposed to DNP+*E. coli*. Inhibition of bacterial transcytosis across metabolically stressed epithelia has been found to be associated with reduced inhibitory protein I-κB phosphorylation and, hence, NF-κB activation (37). The gut microflora in some patients with CD can contain reduced in numbers of butyrate-producing bacteria, and this might result in metabolic stress for the colonocytes. Diets containing fermentable fibers and resistant starches lead to a shift in the host intestinal microbiome, increasing the proportion of butyrate-producing bacteria, and consequently butyrate production. Thus, increasing the butyrate production should also enhance the protection of the epithelium through strengthening the mucosal barrier through increased TJ protein production and TEER, as well as through decreased permeability and bacterial translocation (Figure 2). Similarly, a diet containing probiotic bacteria should in time boost barrier function and integrity. It has therefore been hypothesized that butyrate is important in the maintenance and regulation of the barrier function of the colonic epithelium. It also protects human colon cells from DNA damage through its ability to stimulate the expression of glutathione S-transferases, a multigene family of enzymes mainly involved in the detoxification of chemicals. At a molecular level, butyrate has also been shown to affect gene expression through the phosphorylation and acetylation of histone proteins, particularly

H3 and H4. Hyperacetylation of histones disrupts ionic interactions with the adjacent DNA backbone, creating less densely packed chromatin, or euchromatin, and allowing the transcription factors to activate specific genes (6). Data obtained from studies conducted on Caco-2 cell lines treated with 2mmol/L of butyrate in the culture medium suggest that it may be able to strengthen the tight junction barrier in IBD by increasing the expression of the claudin-2, occludin, cingulin and zonula occludens poteins (ZO-1, ZO-2) (38).

Pathogenesis of IBD: the role of Heat shock proteins

Heat shock proteins (Hsps) are proteic complexes involved in processes like folding, refolding, translocation and degradation of intracellular proteins under normal and stress conditions (39). Hsps are also involved in other processes, such as interactions with the immune system. In fact, when these proteins are released in the extracellular environment, they can trigger an inflammatory response (40), since Hsps are highly conserved molecules that present sequences similar to bacterial and human orthologs (molecular mimicry). Through this mechanism Hsps can stimulate both innate and adaptive immune responses, becoming primary targets of the autoimmune response (41). For this reason, Hsps are thought to be involved in the pathogenesis of a number of chronic inflammatory and autoimmune diseases, such as rheumatoid arthritis, myasthenia gravis, and IBD (42). Hsps have been found to be increased in both tissues and serum of patients with UC, and various mechanisms of action have been hypothesized for these proteins. In a situation of cellular stress (like in IBD), in which the cell needs to produce much more proteins than under normal conditions, Hsps levels increase (since they are involved in the folding of newly generated proteins) may reflect an antipathogenic mechanism that could be used as a biomarker to assess prognosis and response to the treatment. Another mechanism of action may be explained by the molecular mimicry of Hsps with foreign components (such as GroEL in the bacteria of intestinal microflora) that can trigger a response against self Hsps. This situation leads to the formation of antibody-Hsp complexes that can set off the inflammation process typical of IBD (43). Some aspects of the role played in IBD pathogenesis by molecular chaperones interacting with components of the immune system involved in inflammation still remain unclear. Thus, we still need to understand exactly why and how inflammation develops and is maintained through periods of relapse and remission. Hsp chaperones such as Hsp70, Hsp60 and Hsp10 play a

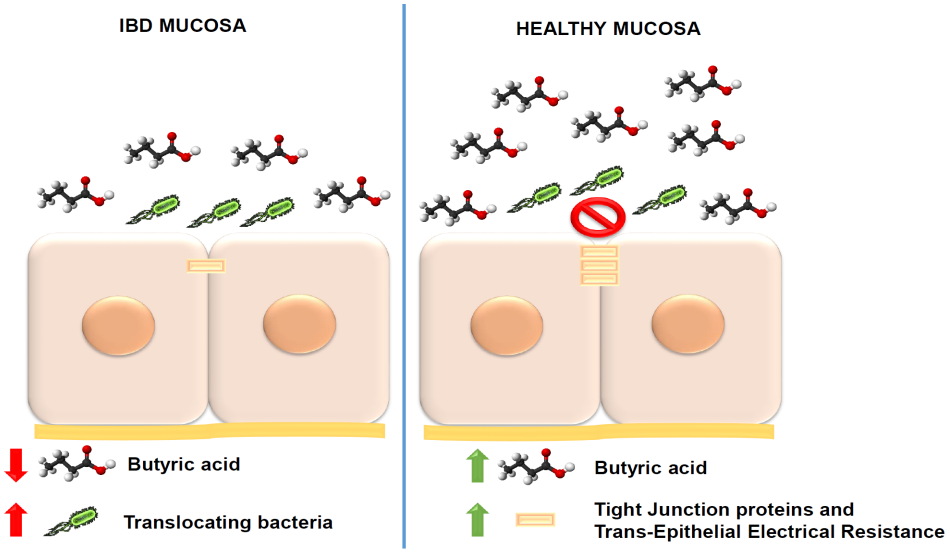

Figure 2: Butyric acid increases TEER through a mechanism that involves an increased production of the proteins that form Tight Junctions. In this way, the permeability of the mucosa is reduced, resulting in decreased translocation of bacteria through the mucosal barrier.

critical role in the inflammatory response to a number of noxae or stressors and in immune regulation (44). It could therefore be useful to revisit CD and UC under the light of this new information in order to fully understand all the aspects of the involvement of Hsp chaperones in these diseases and their role in the pathogenesis of such conditions. The roles of the chaperonins Hsp60 and Hsp10 have already been studied relatively extensively. Both were traditionally considered intracellular molecules, confined to the mitochondria and dedicated to assisting protein folding. However, in the last few years Hsp60 and Hsp10 have been found in other locations outside the mitochondria, such as the cytosol, cell membrane, intercellular space, and blood (45, 46). It has been demonstrated that mucosal Hsp60 decreases in UC patients treated with combination therapies (mesalazine+probiotics), suggesting that this chaperonin could be a reliable biomarker for monitoring response to the treatment, and that it might play a role in the pathogenesis of this disease (47). A study on mucosal biopsies obtained from 40 patients with mildly to moderately active UC without systemic comorbidities analyzed changes in Hsp10, Hsp70 and Hsp90 levels and localization in the epithelium and lamina propria. Samples were obtained at diagnosis and after therapy. Patients were treated for a period of six months with 1.2 or 2.4 g/die (depending on disease severity) of mesalazine (a drug composed of 5-ASA linked to sulfapyridine via a diazo bond that is cleaved by bacterial azoreductases in the colon to release the two components (48)) or with a combination of mesalazine and 1 capsule/die of probiotics (Acronelle®, Bromatech, Milan, Italy). Analysis of Hsp10, Hsp70 and Hsp90 levels and localization was performed with immunohistochemistry. The results showed that Hsp levels changed significantly after therapy: Hsp10 decreased in both the epithelium and the lamina propria in the mesalazine group and the mesalazine+probiotics groups; Hsp70 decreased only in the epithelium of the mesalazine and the mesalazine+probiotics groups, and Hsp90 decreased only in the lamina propria of the mesalazine+probiotics group (49). Thus the levels of Hsp10, Hsp70 and Hsp90 are increased in UC patients at the time of diagnosis and decrease with therapy, supporting the notion that these proteins should be taken into consideration in the study of the mechanisms that promote the development and maintenance of IBD, and as biomarkers of this disease (e.g. to monitor response to treatment at the histological level).

Effects of butyric acid on Hsp expression in colonocytes

Butyric acid is a modulator of growth, function, and differentiation of intestinal epithelia (50). It arises from bacterial fermentation of undigested or malabsorbed dietary carbohydrates. When IECs come into contact with heat, osmotic, oxidative, or other stresses, butyric acid can stimulate the activation of a "stress tolerance" system based on the induction of cytoprotective Hsps. Among these proteins there are Hsp25 (involved in the stabilization of the actin cytoskeleton), Hsp72 (highly stress-inducible) and Hsp73 (constitutive) involved in the prevention of cell denaturation (51). These Hsps help to maintain efficient tight junctions between IECs, thus promoting the function of the mucosa. A study investigating the effects of three different kinds of diets in rats [cellulose free, normal and pectin-enriched (to enhance the production of butyric acid in the colon)] found differences in the genic expression of the three groups, and consequently, in their proteomic assets. Western -blot analysis of Hsp25, Hsp72 and Hsp73 found an increase in the Hsp25 levels in the colonic mucosa derived from rats fed with a pectin-based diet, but no differences were found in the levels of Hsp72 and Hsp73 between the groups (52). Some studies have suggested that the Hsp25-mediated cytoprotective effect involves cytoskeletal stabilization or protection of critical cellular proteins (53). How butyrate and other SCFAs induce Hsp25 expression is not known. Butyrate can inhibit histone deacetylase, causing changes in the histone that may modulate gene transcription rates (54). Butyrate can also alter gene transcription rates through the activation of specific DNA sequences that are putative butyrate response elements (transcriptional binding domains of approximately 11–13 base pairs) (55). A study on rats with a dextran sodium sulfate (DSS)-induced UC treated with butyrate

showed a significant protective effect of the acid against the decrease in cell viability, with an increase in mucosal permeability and polymorphonuclear neutrophil infiltration. It has also been shown that butyrate inhibits Hsp70 expression in DSS colitis, and the activation of HSF (a transcription factor involved in the expression of Hsp genes) and Nf-kB. Butyrate enema was therefore found to be cytoprotective in DSS colitis, an effect partly mediated by suppressing the activation of Hsp70 and Nf-kB (32).

Conclusions

Butyric acid is a SCFA with multiple effects on the physiological activities of colonocytes. Together with all the considerations discussed in this review, this particular clearly shows the importance of studying the metabolic patterns of multifactorial diseases like IBD. In fact, recently it has been considered important to analyze the problems related to human diseases with an integrated approach rather than the traditional reductionist approach used until now. In fact, this can be considered the era of "omic" sciences such as Genomics, Proteomics, Trascriptomics, Interactomics and Metabolomics. Butyric acid derived from bacterial fermentation is used by colonocytes primarily as an energy source, but it can also influence gene expression since it is able to inhibit HDAC. This epigenetic phenomenon can influence the production of proteic factors such as proinflammatory factors (Nf-κB) and Hsps. The latter can promote protective effects (Hsp25 stabilizes the actin cytoskeleton) or interact with the immune system, inducing an autoinflammatory response (like Hsp60). At this time, butyric acid is used as a coadjuvant in the treatment of IBD, since it has shown anti-inflammatory effects on the mucosa of the colon. Moreover, it has been reported that butyric acid is able to induce the production of cytoprotective Hsps (like Hsp25 and Hsp72) and to inhibit the production of some Hsps, such as Hsp70 (that may play a role in the progression of the disease pathogenesis). Butyrate is able to support colonocytes primarily as an energetic substrate, moreover increasing the expression of tight junction proteins and determining an amelioration of the general state of inflamed mucosa in IBD (by decreasing mucosal permeability), in addition to having well documented anti-inflammatory properties. Considering the multiple roles played by butyric acid in the physiology of colonocytes, it may be seen as a valid supplementary therapy for IBD to improve the general condition of the mucosa. This is a highly current topic, subject of continued interest in the international literature. Further clinical trials are necessary to better define the biomolecular dynamics involved in the decrease of intestinal phlogosis in patients with IBD.

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