

Possible Prophylaxes of Aloe Vera Gel Ingestion to Butyrate Metabolism

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ABSTRACT

There is a growing interest in butyrate because its impact on epigenetic mechanisms will lead to more specific and efficacious therapeutic strategies for the presentation and treatment of different disease ranging from genetic/metabolic conditions to neurological degeneration disorders. The dietary natural source of butyrate through a high fiber diet or butyrate produced by fermentation of non-digestive fiber, such as acemannan in *Aloe Vera* leaf gel, is a highly appealing approach to present a simple and relatively low risk method to potentially improve outcomes in aged people with brain troubles. In this review, we will discuss the pharmacological effects of butyrate as a histone deacetylase inhibitor to an insulin resistance and energy expenditure, and as pro-drugs to ulcerative colitis and cancer, and the gut-liver axis in pre-clinical treatment.

Key words: Possible prophylaxes; *Aloe Vera* gel ingestion, Butyrate metabolism

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INTRODUCTION

An importance for understanding the microbial ecology of the gastrointestinal ecosystem and assessing how the microbial world within us impacts on our every day life. Diet metabolism and immunomodulatory activity are linked to both to each other, allowing mammals to adapt to diverse changes in their intestinal gut surroundings. In an earlier report Yagi A. reported that long-term dietary intake of *Aloe Vera* gel influences the structure and activity of the microbial residing in the human gut^[1], and the gut microbiome has played a crucial role in the bidirectional gut-brain axis that integrates the gut and central neurons system activities, and thus the concept of microbiome-gut-brain axis is emerging^[2]. Butyrate in short chain fatty acids (SCFAs) is naturally produced by the gastrointestinal microbiota or orally ingested as a feed additive. Recent data concerning butyrate production delivery as well as absorption by the colonocytes are reported. Butyrate cannot be detected in the peripheral blood, which indicates fast metabolism in the gut wall and/or in the liver.

Cummings JH. group reported that evidence for the occurrence of microbial breakdown of carbohydrate in the human colon has been sought by measuring SCFAs concentrations in the contents of all regions of the large intestine and in portal, hepatic and peripheral venous blood obtained at autopsy of sudden death victims within four hours of death. Total SCFAs ($\mu\text{mol/L}$) in blood was; portal 375 ± 70 , hepatic 148 ± 42 , peripheral 79 ± 22 . In all samples acetate was the principal anion but molar ratios of the three principal SCFAs changed on going from colonic contents to portal blood to hepatic vein indicating greater uptake of butyrate by the colonic epithelium and propionate by the liver. The data obtained indicated that substantial carbohydrate, and possibly protein, fermentation is occurring in the human large intestine, principally in the caecum and ascending colon and that the large bowel may have a greater role to play in diges-

tion^[3]. The effect of butyrate enemas was tested by Scheppach W. and his group, in 10 patients with distal ulcerative colitis (UC) who had been unresponsive to or intolerant of standard therapy for 8 weeks. They were treated for 2 weeks with sodium butyrate (100 mmol/L) and 2 weeks with placebo in random order (single-blind trial). The data obtained support the view that butyrate deficiently may play a role in the pathogenesis of distal UC and butyrate irrigation ameliorates this condition^[4].

The gut microbiome has played a crucial role in the bidirectional gut-brain axis that integrates the gut and central nervous system activities, and thus the concept of microbiome-gut-brain axis is emerging. Nelson ED. group exhibited *Aloe Vera* gel shows positive effects on cognitive function and mood in healthy adults and promises, when taken orally, in supporting neurologic health and function^[5]. In an early *in vitro* study, the fermentation production of hydrophobic butyric acid was confirmed by endophytic bacteria within *Aloe Vera* gel, supporting the possible incorporation of *Aloe Vera* gel as prebiotics aimed at improving gastrointestinal health^[6].

The effects of naturally occurring butyrate-producing probiotic *Clostridium butyricum* CGMCC0313.1 (CB0313.1) were examined by Shang H. group, in limiting the development of high fat diet (HFD)-induced obesity. Mice treated with CB0313.1 exhibited reduced lipid accumulation in liver and serum, lower circulating insulin levels and improved glucose tolerance and insulin sensitivity. CB0313.1, targeting colon inflammation and permeability, ameliorated HFD-induced obesity, insulin resistance as well as adipose inflammation^[7].

Present review summarizes various mechanisms in which butyrate may influence brain health in a number of different neurological disorders. The dietary sources of butyrate through a high-water soluble non-digestive fiber, acemannan in *Aloe Vera* gel, are a highly appealing approach, as butyrate presents a simple and relatively low risk method to potentially improve outcome in patients with gut, hepatic and brain disorders.

A HISTONE DEACETYLASE INHIBITOR, BUTYRATE IN HEPATIC ISCHEMIA/REPERFUSION INJURY

Butyrate, a short chain fatty acid produced by bacterial fermentation of water soluble non-digestive fiber in the colon, has been extensively studied as a histone deacetylase (HDAC) inhibitor. The effect of butyrate administered at the onset of ischemia for HDAC inhibition in hepatic ischemia/reperfusion (I/R) injury was investigated. Sprague Dawley rats were subjected to warm ischemia for 60 min followed by 6 and 24 h of reperfusion. Butyrate was administered at the onset of ischemia. Liver injury was evaluated by serum levels of aminotransferase, inflammatory factors, and histopathology. The study by Sun J. and his group demonstrated that I/R resulted in marked reduction of histone acetylation; butyrate exerted a great hepatoprotective effect through HDAC inhibition and heat shock protein 70 induction^[8]. Incubation of HEK293 or HeLa epithelial cells with SCFAs (butyrate or propionate) at physiological concentrations enhanced NF- κ B activation induced by TLR5, TLR2/1, TLR4, and TLR9 agonists. NF- κ B activation in response to tumor necrosis factor α (TNF α) was also increased by SCFAs. Comparative transcript analysis of HT-29 colon epithelial cells revealed that SCFAs enhanced TLR5-induced transcription of TNF α but dampened or even abolished the TLR5-mediated induction of IL-8 and monocyte chemoattractant protein 1. Bu-

tyrate or propionate caused a rapid increase in histone acetylation in epithelial cells. Lin MY. and his group showed that bacterial SCFAs rapidly alter the epigenetic state of host cells resulting in redirection of the innate immune response and selective reprogramming of cytokine/chemokine expression^[9].

EFFECT OF BUTYRATE TO INSULIN RESISTANCE AND ENERGY EXPENDITURE

To assess the usefulness of measuring butyrate metabolism as an indication of inflammatory activity, Kato K. and his group investigated the rate of butyrate metabolism by breath test after administering [1-¹³C]-butyrate rectally to patients with ulcerative colitis (UC). Thirty-eight UC patients (22 active, 16 quiescent) and 15 healthy controls were given [1-¹³C]-butyrate enemas. The ¹³CO₂ production rate was measured by breath test using an infrared spectrometric analyzer. The ¹³CO₂ production rate was significantly increased in the quiescent stage as compared with the active stage in six UC patients, in whom clinical remission was achieved, in accordance with improvements in the clinical activity index, the endoscopic activity index, histology and fecal butyrate concentrations. Significant inverse correlations between the cumulative ¹³CO₂ production rate and these three parameters were seen in these six UC patients assessed in both the active and quiescent stages. Measurement of expired ¹³CO₂ after rectally administering [1-¹³C]-butyrate in active and quiescent UC appears to be a promising and reliable method for evaluating disease activity and metabolic changes associated with amelioration of inflammation^[10].

Gao Z. and his group examined the role of butyrate in the regulation of insulin sensitivity in dietary-obese C57BL/6J mice. In the obese mice, supplementation of butyrate led to an increase in insulin sensitivity and reduction in adiposity. The mechanism of butyrate action is related to promotion of energy expenditure and induction of mitochondria function^[11].

Sarcopenia, the loss of skeletal muscle mass and function during aging, is a major contributor to disability and frailty in the elderly. Previous studies found a protective effect of reduced histone deacetylase activity in models of neurogenic muscle atrophy. Because loss of muscle mass during aging is associated with loss of motor neuron innervation, Walsh ME. and his group investigated the potential for the histone deacetylase (HDAC) inhibitor butyrate to modulate age-related muscle loss. The authors found significant loss of hind-limb muscle mass in 26-month-old C57BL/6 female mice fed a control diet. Butyrate treatment starting at 16 months of age wholly or partially protected against muscle atrophy in hind-limb muscles. The data show a beneficial effect of butyrate on muscle mass during aging and suggest HDACs contribute to age-related muscle atrophy and may be effective targets for intervention in sarcopenia and age-related metabolic disease^[12].

Henagan TM. and his group investigated the specific genomic loci and mechanisms underlying epigenetically induced obesity and insulin resistance and the targets of sodium butyrate (NaB). NaB treatment may be an effective pharmacological approach for type 2 diabetes and obesity by inducing-1 nucleosome repositioning within nuclear-encoded mitochondrial genes, causing skeletal muscle mitochondrial adaptations that result in more complete β -oxidation and a lean, insulin sensitive phenotype. The authors have provided new mechanistic insight into NaB as an effective pharmacological modulator of obesity and insulin resistance^[13]. Yan H. and Ajuwon K. reported the effects of butyrate during adipogenic

differentiation of porcine stromovascular cells on lipid metabolism and adipokine expression. Differentiating cells were treated with varying concentrations of butyrate. The results show that butyrate treatment enhanced adipogenesis and lipid accumulation, perhaps through upregulation of glucose uptake and de novo lipogenesis. The study presents a novel paradigm for butyrate action in adipocytes and shows that adipocytes are capable of utilizing butyrate, leading to increased expression of adiponectin for enhanced glucose uptake and improved insulin sensitivity^[14].

Obesity is associated with a cluster of metabolic disorders and systemic low-grade inflammation involving multiple organs. Vrieze A. and his group pointed toward a regulating role for butyrate derived from gut microbial metabolism leading to an improvement in insulin sensitivity. First, they observed a significant modification in intestinal microbiota composition in fecal samples after allogenic gut microbiota infusion, including a 2.5-fold increase in the number of bacteria related to the butyrate-producing *Roseburia intestinalis*. Second, they found that bacteria related to the similarly butyrate-producing *Eubacterium hallii* (anaerobic, phylotype *Clostridium* cluster XIVa) were increased in the small intestinal mucosa after allogenic gut microbiota transfer. Butyrate is produced by microbiota both in the large and small intestines for energy and signaling purposes, with orally administered butyrate having a direct effect on glucose metabolism. The authors showed that the effects of infusing intestinal microbiota from lean donors to male recipients with metabolic syndrome on the recipients' microbiota composition and glucose metabolism and intestinal microbiota might be developed as therapeutic agents to increase insulin sensitivity in humans^[15].

Recent findings have suggested that intestine is a key organ altered in response to high fat diet (HFD) feeding. Probiotics mainly *Lactobacillus* strains have been implicated in alleviating metabolic disorders. Shang H. and his group examined the effects of a naturally occurring butyrate-producing probiotic *Clostridium butyricum* CGMCC0313.1 (CB0313.1) in limiting the development of HFD-induced obesity. Mice treated with CB0313.1 exhibited reduced lipid accumulation in liver and serum, lower circulating insulin levels and improved glucose tolerance and insulin sensitivity. Furthermore, CB0313.1 administration reversed the HFD-induced colonic inflammation as evidenced by reduced tumor necrosis factor (TNF)- α level and increases the interleukin (IL)-10 and IL-22 levels in colon tissue. Additionally, to colonic inflammation, CB0313.1 also reduced the colon permeability by upregulating the tight junction proteins (claudin-1 and occludin) and contributed to a decreased circulating endotoxin level. In colon content, CB0313.1 administration restored the reduced production of butyrate and other short chain fatty acids caused by HFD feeding. In adipose tissue, lower transcriptional levels of pro-inflammatory TNF- α , IL-6, IL-1 β and monocyte chemoattractant protein-1 in adipose tissue were observed in CB0313.1-treated mice. Collectively, the authors demonstrated that CB0313.1, targeting colon inflammation and permeability, ameliorated HFD-induced obesity, insulin resistance as well as adipose inflammation^[7].

In the review: possible efficacy of *Aloe Vera* gel metabolites in long-term ingestion to insulin sensitivity, Yagi A. reported the beneficial effects of manno-oligosaccharide in *Aloe Vera* gel to gut-brain (hypothalamus) axis of hepatic glucose production and insulin sensitivity, and proposed an assumption based on a brain-liver-intestine circuit to coordinate with insulin sensitivity of endogenous glucose production^[1].

EFFECTS OF BUTYRATE FROM NEURO-EPIGENETICS AND THE GUT MICROBIOME

Irritable bowel syndrome (IBS) is a common health issue that is characterized by abdominal pain, abnormal bowel movements, and altered visceral perception. Current therapy for IBS is primarily focused on reducing the abdominal pain, thereby improving the quality of life to a significant extent. Although the use of fiber rich diet is widely recommended in treating IBS, some studies have questioned its use. Intra-colonic butyrate is primarily produced by the fermentation of dietary fibers in the colon. In rats it is known to induce visceral hypersensitivity without altered pathology, whereas in humans it has been reported to reduce visceral pain. Understanding the molecular mechanisms responsible for this contrasting effect of butyrate is important before recommending fiber rich diet to IBS patients.

There is a growing interest in butyrate because its impact on epigenetic mechanisms will lead to more specific and efficacious therapeutic strategies for the prevention and treatment of different diseases ranging from genetic/metabolic conditions to neurological degenerative disorders. Berni Canani R. and his group reported review focused on recent data regarding the epigenetic effects of butyrate with potential clinical implications in human medicine^[16].

Kannampalli P. and his group observed from these reports that the dose of butyrate administered plays a critical role in producing the observed effect and hence it is important to know that threshold level of colonic butyrate beyond which it produces hypersensitivity in rats. On the other hand, the results of human experiments necessitates further exploration to know the effect of rectal instillation of butyrate on IBS patients and whether it has any role in modulating visceral pain, since the existing clinical study is restricted only to healthy volunteer^[17].

Sodium butyrate (NaB) is a dietary microbial fermentation product of fiber and serves as an important neuromodulator in the central nervous system. Sun J. and his group investigated NaB attenuated cerebral ischemia/reperfusion (I/R) injury *in vivo* and its possible mechanisms. NaB (5, 10 mg/kg) was administered intra-gastrically 3 h after the onset of re-perfusion in bilateral common carotid artery occlusion mice. The results showed that 10 mg/kg NaB treatment significantly ameliorated neurological deficit and histopathology changes in cerebral I/R injury. Moreover, 10 mg/kg NaB treatment also remarkably inhibited the apoptosis, decreasing the levels of caspase-3 and Bax and increasing the levels of Bcl-2, p-Akt, and brain-derived neurotrophic factor (BDNF). This study suggested that NaB exerts neuroprotective effects on cerebral I/R injury by antioxidant, anti-inflammatory, and antiapoptotic properties and BDNF-PI3K/Akt pathway is involved in anti-apoptotic effect^[18].

Sodium butyrate (NaB) is recognized as a HDAC inhibitor exhibiting anti-inflammatory and neuroprotective effects in a rat ischemic model of stroke as well as a myocardial ischemia model. Although clinical evidence shows that older women are at higher risk for stroke occurrence and greater stroke severity, no studies have evaluated the effectiveness of the HDAC inhibitor either in females or in aged animals. Additionally, few studies have used this treatment at a time point > 6 h following stroke, thus the effectiveness of delayed NaB administration is not well understood. Park MJ. and his group tested the HDAC's neuroprotective effect on middle-aged female rats after ischemic damage. Middle-aged Sprague-Dawley female rats (9-11 months old, constant diestrus) were used for all experiments. Middle cerebral artery occlusion (MCAo) was induced

by intracerebral injection of endothelin-1 (ET-1, 600 pmol). Rats were treated with NaB (300 mg/kg, i.p.) at 6 h and 30 h following ET-1 injection. Treatment with NaB by i.p. injection 6 h after MCAo followed by another injection 30 h later reduced brain infarction determined at 5 days. The effect was consistent both in cortex and striatum with an approximately 40% and 60% decrease, respectively, in infarct volume ($n = 6\sim7$; $p < 0.01$). The protective effects of NaB against MCAo were associated with the anti-inflammatory effect of this HDAC inhibitor. Specially, NaB treatment significantly suppressed MCAo-induced increase of IL-1 β , IL-17A, and IL-18 in brain lysates from the ischemic hemisphere. No significant alterations were observed in cytokines and chemokines in circulating serum level at 5 day post MCAo. These findings support the HDAC inhibitor, NaB, exhibits neuroprotective effects against MCAo-induced brain damage. This is also the first report that NaB treatment post-stroke is neuroprotective in older female rats^[19].

Bourassa MW. and his group reviewed evidence that butyrate produced by bacterial fermentation of fiber in the colon, can improve brain health. Butyrate has been extensively studied as a histone deacetylase inhibitor but also functions as a ligand for a subset of G-protein-coupled receptors and as an energy metabolite. These diverse modes of action make it well suited for solving the wide array of imbalances frequently encountered in neurological disorders. In this review, the authors integrated evidence from the disparate fields of gastroenterology and neuroscience to hypothesize that the metabolism of high fiber diet in the gut can alter gene expression in the brain to prevent neurodegeneration and promote regeneration. The dietary sources of butyrate through a high fiber diet or a diet rich in natural sources of butyrate is a highly appealing approach, as it presents a simple and relatively low risk method to potentially improve outcomes in patients with brain disorders. The mechanisms were proposed for the neuroprotective effects of butyrate and the diseases which may benefit from butyrate treatment or a high fiber diet^[20].

BUTYRATE AS A PRO-DRUG IN CLINICAL TREATMENT

Ulcerative colitis

Butyrate has been shown to increase wound healing and to reduce inflammation in the small intestine. In the colon, butyrate is the dominant energy source for epithelial cells and affects cellular proliferation and differentiation. Butyrate enemas in distal ulcerative colitis and other forms of inflammation is based on the assumption that epithelial energy deficiency may be an important factor in the pathogenesis of these diseases and that the supply of the preferred nutrients, i.e. by raising the luminal butyrate concentration above normal, may ameliorate inflammations. Wachtershauser A. and Stein J. reported that the luminal provision of butyrate may be an appropriate mean to improve wound healing in intestinal surgery and to ameliorate symptoms of inflammatory bowel diseases^[21].

Hallert C. and his group investigated whether ulcerative colitis (UC) patients could safely increase the fecal butyrate level by dietary means. The authors enrolled 22 patients with quiescent UC (mean age, 44 years; 45% women; median time from last relapse, 1 year) in a controlled pilot trial lasting 3 months. The patients were instructed to add 60 g oat bran (corresponding to 20 g dietary fiber) to the daily diet, mainly as bread slices. The mean butyrate concentration over the entire test period was $14 \pm 1 \mu\text{mol/g feces}$ ($p < 0.05$). This pilot study shows that patients with quiescent UC can safely take a diet rich in oat bran specifically to increase the fecal butyrate level. This

may have clinical implications and warrants studies of the long-term benefits of using oat bran in the maintenance therapy in UC^[22].

The effects of physiologically relevant concentrations of butyrate on visceral perception in healthy human eleven subjects were examined in randomized double-blind, placebo controlled cross-over study. Vanhoutvin SA. and his group exhibited the effects of physiologically relevant concentrations of butyrate on visceral perception in eleven human subjects in randomized double-blind, placebo controlled cross-over study. The authors reported that colonic administration of butyrate dose-dependently decreases visceral sensitivity in healthy volunteers^[23].

Hamer HM. and his group examined the effects of butyrate on inflammation and oxidative stress in subjects with chronically mildly elevated parameters of inflammation and oxidation stress. Thirty-five patients with ulcerative colitis (UC) in clinical remission daily administered 60 mL rectal enemas containing 100 mM sodium butyrate ($n = 18$) during 20 days. Before and after the intervention feces, blood and colonic mucosal biopsies were obtained. Butyrate enemas induced minor effects on colonic inflammation and oxidation stress. Although UC patients in remission were characterized by low-grade oxidative stress and inflammation, rectal butyrate enemas showed only minor effects on inflammatory and oxidative stress parameters. (ClinicalTrials.gov.identifier: NCT00696098: Official title: Effects of butyrate on colonic health of patients with diarrhea predominant IBS and UC in remission.)^[24].

Machiels K. and his group examined the predominant microbiota from 127 ulcerative colitis (UC) patients and 87 aged and sex-matched control using denaturing gradient gel electrophoresis (DGGE) analysis. Differences were quantitatively validated using real-time PCR. Metabolites were quantified using gas chromatography-mass spectrometry. Based on DGGE analysis, the microbial signature in Crohn's disease (CD) was not present in UC. Real time PCR analysis revealed a lower abundance of *Roseburia homini* ($p < 0.0001$) and *Faecalibacterium prausnitzii* ($p < 0.0001$) in UC patients compared to controls. Both species showed an inverse correlation with disease activity. Short chain fatty acids (SCFAs) were reduced in UC patients ($p = 0.014$), but no direct correlation between SCFAs and the identified bacteria was found. The composition of the fecal microbiota of UC patients differs from that of healthy individuals and a reduction in *R. hominis* and *F. prausnitzii*, both well-known butyrate-producing bacteria of the Firmicutes phylum was found. These results underscore the importance of dysbiosis in inflammatory bowel disease but suggest that different bacterial species contribute to the pathogenesis of UC and CD^[25]. Banasiewicz T. and his group reported that micro-encapsulated sodium butyrate as a supplemental therapy can reduce the frequency of selected clinical symptoms in patients with irritable bowel syndrome, without significant influence on reducing symptom severity^[26].

Cancer and obesity

Steliou K. and his group reviewed efforts to exploit the potential of butyrate in the clinical treatment of cancer and other medical disorders. The efforts were thwarted by its poor pharmacological properties (short-life and first-pass hepatic clearance) and the multigram doses needed to achieve therapeutic concentrations *in vivo*. Pro-drugs of butyric acid, such as pivanex and tributyrin help mitigate the impediments, but have not been viable as therapeutic agents^[27].

Butyrate have been shown to have anti-proliferative and pro-apoptotic effects on colorectal cancer cells. The metabolic fate of butyrate in the cell is important in determining, whether, it acts

as an HDAC inhibitor or is consumed as a short-chain fatty acid. Clinical trials of several HDAC inhibitors for use as anti-cancer drugs, alone or in combination with other anti-cancer therapeutics, are going on. Kim HJ. and Bae SC. summarized their understanding of the molecular and biological events that underpin the anticancer effects of HDAC inhibitors and the outcomes of recent clinical trials involving these drugs^[28].

In human glioma cells, sodium butyrate (SB) has been shown to inhibit proliferation through modulation of the protein levels of various cell cycle regulators. Kim EH. and his group reported that in TNF-related apoptosis-induced ligand (TRAIL)-resistant glioma cells, co-treatment with nontoxic doses of SB and TRAIL resulted in a marked increase of TRAIL-induced apoptosis. This combined treatment was also cytotoxic to glioma cells over-expressing B-cell lymphoma or extra large, but not to normal human astrocytes, thus offering an attractive strategy for safely treating resistant gliomas^[29].

Mechanisms of primary cancer prevention by butyrate were studied by Scharlau D. and his group. Activation of glutathione S-transferase (GSTs) by butyrate has been studied on mRNA, protein, and enzyme activity level by real-time RT-PCR, cDNA microarrays, Western blotting, or photometrical approaches, respectively. Butyrate had differential effects in colon cells of different stages of cancer development. Because butyrate increased histone acetylation and phosphorylation of ERK in HT29 cells, inhibition of histone deacetylases and the influence on mitogen-activated protein kinase signalling are possible mechanisms of GST activation by butyrate. Functional consequences of this activation include a reduction of DNA damage caused by carcinogens like hydrogen peroxide or 4-hydroxynonenal (HNE) in butyrate-treated colon cells. Treatment of colon cells with the supernatant from an *in vitro* fermentation of inulin increased GST activity and decreased HNE-induced DNA damage in HT29 cells^[30].

Gut commensal microbes shape the mucosal immune system by regulating the differentiation and expansion of several types of T cell. A large bowel microbial fermentation product, butyrate, induced the differentiation of colonic regulatory (Treg) cells in mice. Butyrate induced the differentiation of Treg cells *in vitro* and *in vivo*, and ameliorated the development of colitis induced by adoptive transfer of CD4(+) CD45RB(hi) T cells in Rag1(-/-) mice. Treatment of naive T cell under the Treg-cell- polarizing conditions with butyrate enhanced histone H3 acetylation in the promoter and conserved non-coding sequence regions of the Foxp3 locus, suggesting a possible mechanism for how microbial-derived butyrate regulates the differentiation of Treg cells. Furusawa Y. and his group provided new insight into mechanisms by which host-microbe interactions establish immunological homeostasis in the gut^[31].

Han A. group showed that butyrate oxidation is decreased in cancerous colonocytes compared to non-cancerous colonocytes. The data obtained suggest that colorectal cancer cells decrease butyrate oxidation through inhibition of pyruvate dehydrogenase, which is carnitine-dependent, and provide insight into why butyrate shows selective effects toward colorectal cancer cells^[32].

Rutkowski M. and his group showed that the microbiome does not act in isolation; a patient's genetic background can also greatly influence response to therapy. They revealed that a dominant polymorphism of the gene for the innate immune protein toll-like receptor 5 influences clinical outcomes in cancer patients by changing how the patient's immune cells interact with gut commensal microbes. The authors speculate that many more polymorphisms linked to cancer prognosis may act via microbiome-immune system interactions^[33]. Understanding cancer's relationship with the human

microbiome could transform immune-modulating therapies.

Meijer K. and his group suggested that SCFAs, especially butyrate, show an anti-inflammatory effect and seem to have the potency to prevent infiltration of immune cells from the bloodstream in the adipose tissue. SCFAs, especially butyrate, might be good candidates to evaluate in the fight against obesity-associated and systems inflammation in general^[34].

In our earlier pre-clinical study to patients with type 2 diabetes mellitus (T2DM), aloe high molecular weight fractions (AHM, 0.05 g, three time daily intake for 12 weeks and concurrently continued taking their hypoglycemic medications) exhibited a significant hypoglycemic effect. Fifteen patients (9 males and 6 females, exclusion of morbid obesity BMI > 40 kg/cm²) with T2DM uncontrolled with oral hypoglycemic medication (metoformin 0.5g, twice daily and glibenclamide 5mg, twice daily) were enrolled in the study. AHM containing about 1000 KD was biodegraded by intestinal microbiota into oligosaccharides which inhibit absorption of glucose moiety through gut membrane. Our pre-clinical study suggested that AHM as prebiotics may participate to provide the metabolites, such as short chain fatty acids, to patients in T2DM^[35].

de Goffau MC. group investigated the microbiota in children who have diabetes at an early age. The microbiota of children aged 1-5 years with new-onset type 1 diabetes was compared with the microbiota of age-matched healthy controls by use of a deep global analysis of the gut microbiota composition; phylogenetic microarray analysis. In pairs younger than 2.9 years, the combined abundance of the class Bacilli (notably Streptococci) and the phylum Bacteroidetes was higher in diabetic children, whereas the combined abundance of members of Clostridium clusters IV and XIVa was higher in the healthy controls. Controls older than 2.9 years were characterised by a higher fraction of butyrate-producing species within Clostridium clusters IV and XIVa than was seen in the corresponding diabetic children or in children from the younger age groups, while the diabetic children older than 2.9 years could be differentiated by having an increased microbial diversity. The results from both age groups suggest that non-diabetic children have a more balanced microbiota in which butyrate-producing species appear to hold a pivotal position^[36].

Berni Canani R. started phase 2 and 3 clinical trial with randomized double blind design on 2016; ClinicalTrials.gov. identifier: NCT02721953. Official title: The effects of butyrate on children with obesity.

Gut-liver axis

By using rat models of choline-deficient/L-amino acid-defined-diet-induced nonalcoholic fatty liver disease (NAFLD), Endo H. and his group examined whether Clostridium butyricum MIYAIRI 588; a butyrate-producing probiotic, prevents the progression of pathophysiological changes from steatosis to hepato-carcinogenesis. The favorable changes *in vivo* and *in vitro* experiments caused an obvious decrease in hepatic fibrous deposition, glutathione S-transferase placental form-positive foci development, and hepatocarcinogenesis. The authors established that butyrate-producing probiotic MIYAIRI 588 has beneficial effects in the prevention of NAFLD progression through the intestine/liver axis and systematic signalling activation^[37].

Inflammasomes are large multiprotein complexes that have the ability to sense intracellular danger signals through special nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). They include NLRP3, NLRP4, NLRP6 and absent in melanoma 2. They are involved in recognizing diverse microbial (bacteria, viruses, fungi and parasites), stress and damage signals,

which result in direct activation of caspase-1, leading to secretion of potent pro-inflammatory cytokines and pyroptosis. Bawa M. and Saraswat VA. examined the significance of inflammasomes in relation to the gut microflora and liver, and reviewed the emerging functions of human microbiota in health and liver diseases^[38].

Van der Beek CM. and his group investigated the effect of rectal butyrate administration on short chain fatty acid (SCFAs) interorgan exchange. SCFAs are considered beneficial for colonic health, but high plasma concentrations are potentially harmful. Twelve patients (7 men; age: 66.4 ± 2.0 y; BMI 24.5 ± 1.4 kg/m²) undergoing upper abdominal surgery participated in this randomized placebo-controlled trial. During surgery, 1 group received a butyrate enema (100 mmol sodium butyrate/L; 60 mL; $n = 7$), and the other group a placebo (140 mmol 0.9% NaCl/L; 60 mL; $n = 5$). After colonic butyrate administration, splanchnic butyrate release was prevented in patients undergoing upper abdominal surgery. These observations imply that therapeutic colonic butyrate administration at this dose is safe. The trial was registered at Clinicaltrial.gov as NCT02271802. Official title: Effect of a butyrate enema on the systemic concentration of short chain fatty acids^[39].

The complex interplay between gut microbiota, immune responses, and inflammatory conditions was summarized by Shen S. and Wong CH. as followings: (1) Therapies such as antibiotics and FMTs shift the composition of the whole microbiota, altering the relative abundance of the main phyla Bacteroides and Firmicutes. (2) Other therapies such as probiotics and prebiotics promote the growth and colonisation of selective genus of bacteria, such as Lactobacillus and Bifidobacteria. (3) Prebiotic fiber can also be fermented to SCFAs by certain bacteria. SCFAs, such as butyrate is preferred energy source for colonic epithelial cells, and SCFAs can also modulate immune cell functions. (4) It is now known that the microbiota is not only essential for the development of the immune system, but may also modulate inflammatory responses. (5) Dysbiosis may lead to polarized induction of immune cells. (6) Increased pro-inflammatory T cells may increase inflammatory effector cells, leading to an increased inflammatory state, and may pose as a risk factor for inflammatory diseases, or fuel disease development and severity. On the other hand, induction of regulatory T cells dampens the inflammatory response, and alleviate inflammatory disease phenotype. Finally, expressive inflammation decreases the gut epithelial integrity, which leads to increased bacterial translocation and further induction of inflammation^[40].

CONCLUSION AND FUTURE PERSPECTIVES

Pharmacologically, butyrate is capable of targeting many pathways with multiple mechanisms of action that are disease specific. Butyrate, fermented from *Aloe Vera* gel ingestion might prevent infiltration of immune cells from the bloodstream in peripheral tissues, for example, adipose tissue. Butyrate seems to have an anti-inflammatory effect mediated by signaling pathways like NF- κ B and an inhibition of histone deacetylase (HDAC). The accumulating evidence provides a strong rationale in support of clinical studies to evaluate the safety and therapeutic profile of HDAC inhibitors in combination with anticancer immunotherapy. Further investigations using clinical studies are needed, (1) to examine the gut microbial change in long-term *Aloe Vera* gel ingestion to prevent age-related diseases in elderly; (2) to confirm the effects of acemannan in *Aloe Vera* gel as prebiotics and its metabolites, such as short chain fatty acids; butyrate, to gut-brain (hypothalamus) axis of hepatic glucose production and insulin sensitivity; and (3) to research what would be

the molecular mechanisms underlying the intimate cross talk between the immune system and the microbiota-gut-brain axis at its various nodes of interactions.

Microbiota-gut-brain axis and microbiota-gut-liver axis, and a new link between gut commensal microbiota and distal malignant progression through tumor-promoting inflammation, will gain market traction within the next few years. We can no longer overlook the importance of gut-brain axis, gut-liver axis and butyrate nutrition in disease pathogenesis and treatment.

CONFLICT OF INTEREST

All authors declared no potential conflicts of interest.

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