

Immune Modulation by Microbiota Sources: Effects of *Aloe vera* gel and Butyrate

Akira Yagi, Byung Pal Yu

Akira Yagi, PhD., Professor Emeritus, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Hiroshima, Japan
Byung Pal Yu, PhD., Professor Emeritus, Department of Physiology, University of Texas, Health Science Center, San Antonio, TX, the United States

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Correspondence to: Akira Yagi, Editor-In-Chief, 2-10-1 Hanagaura, Kasuya-machi, Kasuya-gun, Fukuoka-ken, 811-2310, Japan.
Email: 0131akirayagi@gmail.com
Telephone: +81-92-938-2717
Fax: +81-92-938-2717

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ABSTRACT

The role of gut microbiota and its related activities, like fermentation in immune modulation, are well documented. It was found that acemannan (AM), the extract of aloe leaf gel can exerts significant immune modulating activity. AM has been shown to activate macrophages and stimulate T cells that are involved in the defense against harmful microorganisms by likely acting as an adjuvant for health and disease. However, it should be noted that commercially available AM preparation used for these studies often contains a complex mixture because partially purified carbohydrate preparation containing about 60% acetylated mannan together with other carbohydrates, especially pectin, hemicellulose, and lectins.

The beneficial effects of endogenous butyrate productions, delivery, and absorption by colonocytes have been well documented. Mechanistically, butyrate exerts its functions by acting as a histone deacetylase inhibitor or signaling through several G-protein-coupled receptors. Recently, butyrate has received particular attentions for its beneficial effects on intestinal homeostasis and energy metabolism.

Key words: Immune modulation; *Aloe vera* gel; Butyrate

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INTRODUCTION

Dietary fiber is the primary energy source for maintenance of a healthy bacterial community and source of bioactive fermentation metabolites, such as short chain fatty acids (SCFAs), including butyrate in particular, which are important for attenuating colonic and systemic inflammation for human health. In previous paper, we proposed a novel immune-enhancing complex carbohydrate, AM in aloe vera gel and its importance for microbiota-inducing gut immunity^[1]. Carbohydrates play critical roles in immune system function. Some carbohydrates from plant, bacteria, yeast have emerged as promising vaccine adjuvant candidates. AM is a partially purified carbohydrate preparation, contained about 60% acetylated mannan together with pectin, hemicellulose, and lectin. AM has been claimed to possess many, if not all of the important biological activities of the aloe vera pulp. However, it is known that technically difficult to separate AM from contaminating pectin, hemicelluloses, proteins, and lectins. AM can activate macrophages. This macrophage activity as an adjuvant is responsible for the pro-wound healing activity, as well as its anti-tumor, and anti-viral activity.

A pilot study was undertaken by Yates *et al*^[2] to determine AM's effect in 49 feline immunodeficiency virus (FIV) infected cats with clinical signs of disease (stage 3, 4 or 5), 23 of which had

severe lymphopenia. Ali *et al.*^[3] investigated the effect of dietary supplementation with aloe vera powder in broilers. AM was evaluated as an immune-modulatory in cats and chickens. AM was approved by the United States Department Agriculture for use as a vaccine adjuvant in chickens, cats and dogs.

McDaniel *et al.*^[4,5] studied a 6 year analysis on 5 survivors of a 1986 open-labeled clinical pilot study to evaluate the potential efficacy of oral AM 800 mg/day in the treatment of symptomatic HIV-1 infected patients. The results showed that AM may potentiate the antiviral drug azidothymidine (AZT). Researchers claimed that the use of AM may reduce the amount of AZT required by as much as 90%. The further confirmation of efficacy of AM in clinical settings would be an important therapeutic advancement. The pilot studies strongly suggest that the importance for understanding the microbial ecology of the gastrointestinal ecosystem and how the microbial world within patients impacts on the efficacy of A M.

In the present paper, we describe immune modulation by microbiota and its related activities, like gut fermentation of aloe vera gel and butyrate by reviewing some pre-clinical studies on health and disease status including type 2 diabetes.

IMPORTANCE OF MICROBIOTA FOR HEALTH AND DISEASE

While there is plenty of evidence showing that host's genetics influences and interacts with gut microbiota, epigenetic alteration of gut microbiome and metabolites produced by gut microbiota as the possible causes of various diseases. Thus, a better understanding of the cellular process involved in the homeostatic interaction between the gut microbiota and the host can facilitate the revelation of new approaches for the treatment of diseases and the maintenance of health.

Aloe vera gel as immune-regulatory materials

Many non-starch polysaccharides (NSPs) classified as dietary fibers have been reported by Flint HJ^[6] to possess immune-regulatory properties. The fibers reported to activate or by other means modulate immune responses originate from both plant, fungal, and microbial sources and constitute highly distinct structures. As many of the preparations were tested for constituent crude extract or partly purified NSPs, the risk of contaminants holding immune-regulatory activities should not be ignored.

A double-blind, randomized, placebo-controlled clinical trial of the efficacy and safety of aloe vera gel for the treatment of mildly to moderately active ulcerative colitis, was performed by Langmead *et al.*^[7]. Forty-four hospital out-patients were randomly given oral aloe vera gel or placebo, 100ml twice daily for 4 weeks, in a 2:1 ratio. Clinical remission, improvement and response occurred in nine (30%), 11 (37%) and 14 (47%), respectively, of 30 patients given aloe vera, compared with one (7%), one (7%) and two (14%), respectively, of 14 patients taking placebo. The Simple Clinical Colitis Activity Index and histological scores decreased significantly during treatment with aloe vera, but not with placebo. Sigmoidoscopic scores and laboratory variable showed no significant differences between aloe vera and placebo. Adverse events were minor and similar in both groups of patients. In conclusion, oral aloe vera taken for 4 weeks produced a clinical response more often than placebo, and also reduced the histological disease activity without showing any harmful effects. Storsrud *et al.*^[8] investigated the effect of aloe vera extract (AVH200) in adult patients with irritable bowel syndrome (IBS) in a randomized,

double-blind, placebo controlled clinical study. Sixty-eight adult patients diagnosed with IBS according to the Rome III criteria were randomized to receive AVH200 or matching placebo for four weeks. Symptom questionnaires were completed on a weekly basis and the patients were asked if they had adequate relief of their gastrointestinal symptoms. It was concluded that even though the primary endpoint was not met, AVH200 seems to be a promising treatment option for patients with IBS because of the positive results seen within the secondary endpoints. Avjigan *et al.*^[9] compared the effectiveness and cost of aloe vera gel with conventional treatments in patients with chronic ulcers. This clinical study was conducted on 60 patients with chronic ulcers (more than 3 weeks) in Al-Zahra hospital in 2015. The participants were divided into two groups of 30 patients per group. In one group, conventional treatment plus aloe vera gel and in the other group, only the conventional treatment was used for 3 months. The overall mean time of wound healing was 31.25 ± 11.2 and 63.2 ± 20.4 in the aloe vera gel and control groups, respectively ($p < 0.05$). The mean hospitalization time was 35.2 ± 6.4 and 67.4 ± 8.9 in the aloe vera and control groups, respectively ($p < 0.05$). These data indicate that aloe vera gel is beneficial and cost effective for patients with chronic ulcers.

In our previous paper, we isolated glycoprotein fraction (verectin) containing a proliferation-promoting activity on human and hamster cells from aloe vera gel freeze-dried powder^[10]. Verectin inhibited Cox-2 and thromboxane A₂ synthase level, suggesting strongly the participation to the immune inflammatory system^[11]. Furthermore, the verectin-derived N-terminal octapeptide (Asp-Glu-Asp-Asn-Val-Leu-Leu-Thr) exerted a significant in vitro inhibitory effect on the growth of cell numbers of Ehrlich ascites carcinoma, which was correlated well with the prolongation of life span of the tumor transplanted mouse^[12].

Extraction, purification, and gel preparation of aloe vera pectin and the evaluation of biocompatibility of the pectin gels were studied by Gentilini *et al.*^[13]. Cytocompatibility of pectin gels, prepared by inotropic gelation showed an improved cell adhesion and high rhamnose content matrices for application in regenerative medicine. In our previous study, we showed that a $\beta 1 \rightarrow 4$ -linked D-mannopyranose containing 18% acetyl groups isolated from the pulp of *Aloe saponaria*, inhibited carrageenan-induced hind paw edema at 50 mg/kg intraperitoneally in rats. The acetyl D-mannopyranose was effective when given intraperitoneally, but not when given orally, suggesting that the effect may be participated with metabolism in gut^[14]. On the metabolism of aloemannan (neutral polysaccharide fraction: ALM) in our previous study, the catabolites of ALM showed hexosamine peaks on high performance anion exchange chromatography. The findings suggest that the immunomodulation of ALM may come from not only neutral polysaccharides but also contaminated hexosamine in ALM and/or ALM catabolites^[15]. These studies demonstrated in vivo effects of carbohydrate on microbial infections strongly indicate the underlying immunoregulatory mechanisms.

Influence of Aloe vera gel on anti-virus infection

According to the research at Baylor College of Dentistry in Dallas, TX, dermal patch containing AM hydrogel reduced healing time as well as pain associated with the Canker sores. The AM patch has received FDA approval of OTC drug^[16,17]. Furthermore, Bhalang *et al.*^[18] reported that AM can be used for the treatment of oral aphthous ulceration in patients who wish to avoid the use of steroid medication, although the effectiveness was not comparable to that of 0.1% triamcinolone acetonide.

A preliminary clinical trial of aloe vera gruel on HIV infection was performed by Olatunya *et al.*^[19] with 10 young women patients compared with those of 20 age-matched controls who were on antiretroviral drugs. Their CD4 counts, general improvement, and physical well-being were monitored over a 1-year period. The results suggested that consumption of aloe vera gel helped HIV-infected individuals in the tropics, given its availability and inexpensiveness. Herpes simplex virus (HSV) infection is one of the most common and debilitating oral diseases. Rezazadeh *et al.*^[20] evaluated the anti-HSV-1 activity of aloe vera gel in Vero cell line. The authors showed significant inhibitory effect of 0.2-5% aloe vera gel on HSV-1 growth in Vero cell line, and suggested a useful topical treatment for oral HSV-1 infections without any significant toxicity. Shokraneh *et al.*^[21] investigated the effect of water supplementation of *Aloe vera* as antibiotic growth promoters (AGPs) on performance, intestinal microflora, and immune responses of broilers. Vaccines against influenza disease and sheep red blood cell were administered to immunological stimuli. The populations of *Lactobacilli* spp. and coliforms were enumerated in ileum. The findings demonstrated a possibility of supplementing broiler drinking water with 1% aloe vera gel as an alternative for AGP substitution. Dzielulska *et al.*^[22] investigated the effectiveness of various doses of aloe vera gel and licorice extracts on the course of experimental pigeon paramyxovirus serotype-1 (PPMV-1). The experiment was performed on pigeons divided into 5 groups, which were orally administered aloe vera gel or licorice extracts at 300 or 500mg/kg body weight (BW) for 7 days after experimental inoculation with PPMV-1. The inhibitory effect was observed in pigeons receiving aloe vera extract at 300mg/kg BW, for which PPMV-1 RNA copy numbers were approximately 7-fold lower in brain, 9-fold lower in kidney, and 14-fold lower in liver than those in the control groups.

Aloe vera gel as novel prebiotics

In our previous study the inner leaf gel containing acemannan (AM) in *Aloe vera* facilitates fermentation with endophytic bacteria and butyric acid was identified in ether extract from the gel fermentation broth by GC/MSD analysis^[23]. The endophytic microbiota of aloe vera gel in the fermented media were examined by use of matrix-assisted laser desorption ionization-time of flight mass spectroscopy. The following microbial were identified: *Bacillus cereus*, *B. licheniformis*, *Lactobacillus paralimentarium*, and *Clavispora lusitaniae*^[24]. Furthermore, the prebiotic activity of aloe vera juice with *Lactobacillus fermentum* in in vitro fermentation identified as acetic, propionic and lactic acid^[25]. An innovative concept of symbiosis based on a combination of aloe vera gel containing AM with *L. fermentum*, seems promising for the future intestinal health endeavors.

Zhang *et al.*^[26] performed a systematic search on aloe vera through PubMed, Embase, and Cochrane Central Register of Controlled Trial (RCT) up to January 2016. A total of five RCTs involving 415 participants were included. Compared with the controls, aloe vera supplementation significantly reduced the concentrations of fasting blood glucose (FBG), glycosylated hemoglobin A1c (HbA1c), triglyceride, total cholesterol, and low in increasing serum high density lipoprotein-cholesterol (HDL-C) levels. The evidence from RCTs showed that aloe vera effectively reduce the levels of FBG, HbA1c, triglyceride, TC and LDL-C, and increase the levels of HDL-C on prediabetes and early non-treated diabetic patients.

Carbohydrates such as AM and fructan are among the molecules responsible for tolerating water deficit in other plant species.

Salinas *et al.*^[27] investigated the effect of water deficit on fructan composition and structure in aloe vera grown under water stress. *Aloe vera* synthesizes fructans with a more branched structure, the neofructans and the protective role of neofructans under extreme water deficient was reported. Furthermore, the authors^[28] found fructans induce an increase in the population of *Bifidobacterium* spp. and produce great amounts of short chain fatty acids. Chioldelli *et al.*^[29] evaluated the effects of different extract of *Aloe vera* and *A. arborescens* in fermented milk, taking into account both the prebiotic effect of aloe polysaccharides and the antimicrobial activity of several secondary metabolites. The results demonstrated a beneficial effect of 5% aloe inner gel on *Lactobacillus* growth and confirmed the antimicrobial activity of the phenolic compounds of green rind extracts.

Influence of butyrate on anti-inflammation and antioxidant action

Short chain fatty acids (SCFAs) are bacterial metabolites that mediate the interaction between the diet, the microbiota and the host. Many studies^[30] have indicated that SCFAs exhibit a variety of functions from immune regulation to metabolism in a variety of tissues and organs, and therefore have both a direct and indirect influence on our bodies.

Jahns *et al.*^[31] investigated the effect of a butyrate treatment on catalase and superoxide dismutase (SOD2) in matched human colon tissues of different transformation stages *ex vivo*. Despite a significantly lowered SOD2 transcript and to a lesser extent of protein level after butyrate exposure of normal colon cells, the catalytic activity was significantly enhanced, suggesting an increased protection against tissue superoxide radicals. In malignant tissues, greater variations in response to butyrate were observed. Furthermore, both enzymes showed an age-dependent decrease in activity in normal colon epithelium. Butyrate exhibits potential antioxidant features *ex vivo*. Ranganna *et al.*^[32] explored whether the antioxidant effect of butyrate contributes to anti-proliferation action on vascular smooth muscle cells (VSMC) via modulation of inflammatory response, using western blotting and immunostaining methods. Several observations suggest a link between antioxidant effect and anti-inflammatory response in butyrate arrested VSMC proliferation and emphasize the athero-protective and therapeutic potential of butyrate in vascular proliferative diseases. Boets *et al.*^[33] quantified the fraction of colonic administered SCFAs that could be recovered in the systemic circulation, the fraction that was excreted via the breath and urine, and the fraction that was used as a precursor for glucose, cholesterol and fatty acids. The systemic availability of SCFAs and their incorporation into biologically relevant molecules was quantified. The systematic availability of colonic-administered acetate, propionate and butyrate was 36, 9, and 2%, respectively. Conversion of acetate into butyrate (24%) was the most prevalent interconversion by the colonic microbiota and was not related to the butyrate-producing capacity in the fecal samples. Such information is essential for a better understanding of the molecular mechanisms by which SCFAs beneficially affect physiological functions such as glucose and lipid metabolism and immune function.

Li *et al.*^[34] investigated the effect of butyrate on appetite and energy expenditure, and evaluated the extent of these two components contributing to the beneficial metabolic effects of butyrate. Chronic butyrate supplementation prevented diet-induced obesity, hyperinsulinaemia, hypertriglyceridaemia and hepatic steatosis, largely attributed to a reduction in food intake. Butyrate also modestly

promoted fat oxidation and activated brown adipose tissue (BAT), evident from increased utilization of plasma triglyceride-derived fatty acids. These effects were not due to the reduced food intake, but explained by an increased sympathetic outflow to BAT. Sub-diaphragmatic vagotomy abolished the effects of butyrate on food intake as well as the stimulation of metabolic activity in BAT. Thus, butyrate seems to act on the gut-brain neural circuit to improve energy metabolism via reducing energy intake and enhancing fat oxidation by activating BAT. Yuan *et al.*^[35] found the effects of SCFAs on the activation of Nod-like receptor pyrin domain 3 (NLRP3) inflammasome in endothelial cells and associated carotid neointima formation. Butyrate may have beneficial effects against vascular inflammation or atherosclerosis by its inhibitor action on O_2^- production and consequent NLRP3 inflammasome formation and activation. The results indicated that SCFAs have differential effects on endothelial NLRP3 inflammasome activation and associated carotid neointima formation.

Health benefits of butyrate on autism spectrum disorders (ASD)

Multiple beneficial effects of butyrate on intestinal and extra-intestinal level have been demonstrated. The mechanisms of action of butyrate are different and many of these involve an epigenetic regulation of gene expression through the inhibition of histone deacetylase. The impact on epigenetic alterations should lead to more specific and efficacious therapeutic strategies for the prevention and treatment of various diseases, ranging from genetic/metabolic conditions to neurological degenerative disorders. Canani *et al.*^[36] reviewed published data on the epigenetic effects of butyrate on potential clinical implications in medicinal treatment. Furthermore, Canani *et al.*^[37] demonstrated a genotype-dependency for butyrate therapeutic efficacy in congenital chloride diarrhea (CLD). The authors demonstrated that butyrate may act on either fecal ion loss or the severity of diarrhea in a subset of CLD patients.

Microflora has a substantial influence on energy utilization in the colon compared with other tissues. This tissue specificity is due to colonocytes utilizing bacterially produced butyrate as their primary energy source. Donohoe *et al.*^[38] demonstrated that butyrate is a mitochondrial fuel that is directly integrated into energy metabolism through the production of acetyl-CoA, the first metabolite of the TCA cycle. Germ-free mice demonstrate a specific deficit in bioenergetics in colon tissue with this deficiency rescued by the butyrate-producing bacteria *Butyrivibrio fibrisolvens*. The mechanism in this case is likely that butyrate acts as an energy source rather than as histone deacetylase (HDAC) inhibitor.

Butyrate positively modulates mitochondrial function, including enhancing oxidative phosphorylation and β -oxidation and has been proposed as a neuro-protectant. Butyrate has been associated with autism spectrum disorders (ASD), a condition associated with mitochondrial dysfunction. Rose *et al.*^[39] evaluated mitochondrial function in rectal and cecum biopsies based on the assumption that certain microbiome metabolites, such as butyric and propionic acid, are more abundant in the cecum as compared with the rectum. Mitochondrial function in the gut mucosa from children with ASD was found to be significantly different than other groups who manifested similar gastrointestinal (GI) symptomatology, suggesting a unique pathophysiology for GI symptoms in children with ASD. Interestingly, abnormalities localized to the cecum suggest that the imbalance in the microbiome might play a role in the production of butyrate in children with ASD. Rose *et al.*^[40] developed a lymphoblastoid cell line (LCL) model of ASD, with one subset of LCLs demonstrating mitochondrial dysfunction and

the other subset of LCLs with normal mitochondrial dysfunction. The authors demonstrated that butyrate has a modulatory effect on mitochondrial function in LCLs from both healthy children and children with ASD, and found that butyrate has a positive impact on a cell line under physiological stress. This ability to rescue cells under physiological stress by butyrate may provide insight into the therapeutic potential effect of butyrate for many other diseases associated with mitochondrial dysfunctions.

DIVERSE ROLES OF BUTYRATE IN HEALTH AND DISEASES

Recent data revealed the close interplay between immune and bone cells, gut microbiota (GM) that plays a central role in maintaining bone health and influences bone turnover and density. GM can improve bone health increasing calcium. Bone-related prebiotic effects exist across the lifecycle, suggesting benefits for attainment of peak bone mass during adolescence and minimized bone resorption among postmenopausal women. These effects are thought to occur through prebiotic-microbe interactions in the large intestine. Current prebiotic mechanisms for improved mineral absorption and skeletal health include alterations in GM composition, production of short chain fatty acids (SCFAs), altered intestinal pH, biomarker modification, and immune system regulation. SCFAs have broadly been proposed as key mediators on the host. They are produced by bacterial fermentation of otherwise indigestible nutrients. The most abundant SCFAs are propionate, acetate and butyrate. All have been extensively studied in murine models for their possible influence on colonic health, glucose and lipid metabolism, as well as appetite and energy expenditure. Although various mechanisms by which these SCFAs exert their effect were suggested, the precise pathways remain unclear.

Fluitman *et al.*^[41] identified baseline microbial composition as an essential factor for the response to microbiota fecal transplantation. The highlights are followings: lean donor fecal microbiota transplantation (FMT) in obese metabolic syndrome male subjects improves insulin sensitivity; beneficial effects of lean donor FMT are transient; improvement in insulin sensitivity is linked to changes in plasma metabolites; response to lean donor FMT is driven by baseline fecal microbiota composition. Endogenous butyrate production, delivery, and absorption by colonocytes have been well documented. Butyrate exerts its functions by acting as a histone deacetylase inhibitor or signaling through several G-protein-coupled receptors.

Jiao *et al.*^[42] used a meta-analysis to obtain an unbiased evaluation of structural and functional changes of gut microbiota in diet-induced obese rodents. The raw sequencing data of nine studies generated from high-fat diet (HFD)-induced obese rodent models were processed with Quantitative Insights into Microbial Ecology to obtain gut microbiota compositions. Differential functional pathways of the gut microbiome in obese rodents included enriched pyruvate metabolism, butanoate metabolism, propanoate metabolism, pentose phosphate pathway, fatty acid biosynthesis, and glycerolipid metabolism pathways. These pathways converge in the function of carbohydrate metabolism, SCFA metabolism, and biosynthesis of lipid. HFD-induced obesity results in structural and functional dysbiosis of gut microbiota. The altered gut microbiome may contribute to obesity development by promoting insulin resistance and systemic inflammation.

The modulation of the immune system by butyrate can be exhibited in various ways. For instance, Eftimiadi *et al.*^[43] observed

that addition of butyrate to the culture of immune cells had a double edged effect; Butyrate blocks the development of new immune cells participating in inflammation. Butyrate stimulates the production of several key inflammatory mediators. Butyrate has been shown to exhibit the protective effects against inflammatory diseases such as ulcerative colitis and inflammation-mediated colorectal cancer.

Donohoe *et al.*^[44] looked into the influence of aerobic metabolism that underlies butyrate-induced histone acetylation and cell proliferation. Normal colonocytes utilize butyrate as a major energy source because butyrate enters mitochondrial β -oxidation and yields ATP. In contrast, because of the Warburg effect, cancerous colonocytes metabolize glucose over butyrate resulting in butyrate accumulation in the nucleus. In the nucleus, butyrate regulates genes that reduce proliferation and increases apoptosis as HDAC inhibitor. The findings indicate that butyrate has opposing effects on the growth of normal versus cancerous colonocytes.

Zimmerman *et al.*^[45] showed that butyrate reduces colonic inflammation based on their cell culture system in which butyrate suppresses the activity of cells and proteins deriving inflammation. Butyrate delivers a double-hit: induction of T-cell apoptosis to eliminate the source of inflammation and suppression of IFN- γ -mediated inflammation in colonic epithelial cells, to suppress colonic inflammation. The anti-inflammatory properties may be partly connected to the ability of butyrate to support the development of specific immune cells that block inflammation in the lining of the end gut in mice. Furusawa *et al.*^[46] showed that butyrate generated from a large bowel microbial fermentation can induce the differentiation of colonic Treg cells in mice. These authors provided further insights into the mechanisms by which host-microbe interactions establish immunological homeostasis in the gut.

Most recently, Liu *et al.*^[47] published an interesting review on butyrate's role for its beneficial effects on intestinal homeostasis and energy metabolism. Some of the beneficial metabolic effects of butyrate are mediated through a gut-brain neural circuit to increase insulin sensitivity and glucose tolerance. With anti-inflammatory properties, butyrate enhances intestinal barrier function and mucosal immunity. A number of studies have associated obesity with altered gut microbiota, although results are discordant regarding compositional changes in the gut microbiota of obese animals. The precise role of butyrate in obesity remains controversial. The abnormalities in glycolipid metabolisms are a main reason for obesity, diabetes, and other metabolic syndromes. The current research related to the novel genes regulating glucose and lipid metabolism, which enable us to develop more efficient means of prevention and management of metabolic diseases such as T2D, obesity, high blood glucose, and hypertension^[48]. Ma Xi groups^[47,48] reviewed the present status on the properties of butyrate, especially its potential effects and mechanisms involved in intestinal health and obesity, and the impact of butyrate on glycolipid metabolism abnormalities and disease via the gut-brain-axis.

It seems clear that high-fat diets cause obesity, at least in part, by modifying the composition and function of the microbiota that colonize in the gastrointestinal tract. SCFA promotes colonic integrity, blood-brain barrier integrity and reduces a neuroprotective and anti-inflammatory state in microglia by inhibiting HDAC via the G-protein coupled receptor 43. Moreover, both microbiota and SCFA interact with vagal afferent nerves, communicating with the hypothalamus about inflammation and energy homeostasis. Mulders *et al.*^[49] presented that the potential of intestinal microbiota to induce obesity and multiple ways to modify its composition

and function are investigated to provide novel preventive and therapeutic strategies against diet-induced obesity. In all, it seems clear that microbe-derived butyrate plays an important role in both gut health and obesity of the host.

PRE-CLINICAL STUDIES OF BUTYRATE

Butyrate is important for creating tolerance in the gut and promoting an anti-inflammatory environment. Gut butyrate is mostly not absorbed rather it is primarily utilized by colon cells as a major energy source. Butyrate-producing bacteria live in the end part of the gut in the colon. In the mitochondria of colon cells, 70% to 90% of butyrate is oxidized into acetyl-CoA, which is subsequently processed through the TCA cycle to generate a large quantity of ATP. In the following sections, the various action of butyrate to colorectal cancer, diverticulosis, HIV, schizophrenia, and diabetes in type 2 will be described below.

Inflammation and oxidant status in the colonic mucosa of patients with ulcerative colitis (UC) and crohn's disease (CD)

Rectal enemas containing a short chain fatty acid (SCFA) mixture, butyrate alone, or saline placebo were administered to 47 patients with active UC as reported by Scheppach *et al.*^[50]. After eight weeks, fewer colonic segments were affected endoscopically following butyrate treatment than placebo group. This study showed trends towards a beneficial effect of topical SCFAs in active UC. In addition, the authors^[51] observed that butyrate reduces the density of polymorphonuclear leucocytes in the lamina propria, while other inflammatory parameters remained unchanged. Both butyrate and the SCFA mixture reduced significantly the number of proliferating cells in the upper 40% of crypts. SCFAs and butyrate have a more marked effect on crypt cell proliferation than on parameters of inflammation in patients with active UC. Hamer *et al.*^[52] performed a randomized, double blind, cross-over study with 16 healthy volunteers. Treatments consisted of daily rectal administration of a 60 ml enema containing 100 mM sodium butyrate or saline for 2 weeks. Butyrate treatment resulted in significantly higher GSH and lower uric acid concentrations compared with placebo group. Their study demonstrated that butyrate is able to beneficially affect oxidative stress in the healthy human colon. Clinical trials registration (NCT00693355). Furthermore, the authors^[53] assessed the effects of butyrate on inflammation and oxidative stress in subjects with mildly elevated parameters of inflammation and oxidative stress. Clinical trials registration (NCT00696098). Machiels *et al.*^[54] evaluated in a large cohort whether the microbial signature described in CD is also present or not in UC, and whether the authors could characterize predominant dysbiosis in UC. The predominant microbiota from 127 UC patients and 87 age and sex-matched controls was analyzed using denaturing gradient gel electrophoresis analysis. Real time PCR analysis revealed a lower abundance of *Roseburia hominis* and *Faecalibacterium prausnitzii* in UC patients compared with controls. Both species showed an inverse correlation with disease activity. SCFAs were reduced in UC patients, but no direct correlation between SCFAs and the identified bacteria was found. Interestingly, the composition of the fecal microbiota of UC patients differs from that of healthy individuals: the authors revealed a reduction in *R.hominis* and *F.prausnitzii*, both well-known butyrate-producing bacteria of the *Firmicutes* phylum. These results underscore the importance of dysbiosis in inflammatory bowel disease but suggest that different bacteria species contribute to the pathogenesis of UC and CD.

Laserna-Mendieta *et al.*^[55] performed a comparative assessment of the capacity of the microbiota for butyrate synthesis, by quantifying butyryl-CoA: acetate CoA-transferase (BCoAT) gene content in stool from patients with crohn's disease ($n = 71$), ulcerative colitis ($n = 58$) and controls ($n = 75$), and determined whether it was related to active vs inactive inflammation, microbial diversity, and composition and/or dietary habits. Reduced butyrate-synthetic capacity of the microbiota is more evident in CD than UC and may relate to reduced fiber intake. The results suggest that simple replacement of butyrate per se may be therapeutically inadequate, whereas manipulation of microbial synthesis, perhaps by dietary means, may be more appropriate, as reduced butyrate and low fiber intake are linked to CD.

Altered gut microbiota in colorectal cancer (CRC)

Wang *et al.*^[56] investigated fecal bacterial diversity in CRC patients ($n = 46$) and healthy volunteers ($n = 56$) were profiled by 454 pyrosequencing of the V3 region of the 16S ribosomal RNA gene. Reduction of butyrate producers and increase of opportunistic pathogens may constitute a major structural imbalance of gut microbiota in CRC patients.

The beneficial role of butyrate for patients with diverticulosis

A role of micro-encapsulated butyrate (MB) in patients with diverticulosis, hypothesizing its potential for reduction of diverticulitis episodes and diverticulitis prevention was investigated by Krokowicz *et al.*^[57]. Seventy-three patients with diverticulosis were recruited for the study and randomized. After 12 months, the study group noted a significantly decreased number of diverticulitis episodes in comparison with the control group. The subjective quality of life in the study group was higher than in the control group. There were no side effects of the MB during the therapy. MB reduces the frequencies of diverticulitis episodes, is safe, and improves the quality of life. It can play a role in the prevention of diverticulitis.

Butyrate production and immunity in HIV-infected subjects

Serrano-Villar *et al.*^[58] studied that a deeper understanding of how nutritional interventions could ameliorate gut dysbiosis. Forty-four subjects, including 12 HIV⁺ viremic untreated (VU) patients, 23 antiretroviral therapy-treated (ART⁺) virally suppressed patients (15 immunological responders and 8 non-responders) and 9 HIV⁻ controls (HIV⁻), were blindly randomized to receive either prebiotics (scGOS/lcFOS/ glutamine) or placebo over 6 weeks in this pilot study. Significantly, declines in indirect markers of bacterial translocation and T-cell activation, improvement of thymic output, and changes in butyrate production were observed. Increases in the abundance of *Faecalibacterium* and *Lachnospira* strongly correlated with moderate but significant increases of butyrate production and amelioration of the inflammatory biomarkers soluble CD14 and high-sensitivity C-reactive protein, especially among VU patients. Hence, the bacterial butyrate synthesis pathway holds promise as a viable target for interventions.

Pre-clinical trial of butyrate for improving cognitive function in schizophrenia (SZ)

The short chain fatty acids (SCFA) such as acetate, butyrate and propionate) mediate multiple immune and metabolic pathways. Interestingly, these mediators are often linked to an attenuated lifespan in SZ^[59]. Accumulating data in literature strongly indicate that SCFA can cross the blood brain barrier and target key

inflammatory and metabolic pathways.

Joseph *et al.*^[60] highlighted enriching dietary intake for SCFAs as a potential adjunctive therapy for people with SZ. Li *et al.*^[61] evaluated the efficacy of butyrate as a novel treatment for cognitive deficits in SZ, and proposed phase 2 and 3 study in 2017 (NCT03010865). Chronic high-fat diet consumption caused not only obese-insulin resistance, but also cognitive decline and microglial hyperactivity.

Role of butyrate and inulin supplementation in type 2 diabetic patients

The global incidence of obesity and type 2DM is widely recognized as one of the most challenging threats to public health.

Yimam *et al.*^[62] demonstrated that aloesin containing in *Aloe* species inner gel shows significant impact in reducing glycosylated hemoglobin, fasting blood glucose, fructosamine and plasma insulin level in humans.

Zhang *et al.*^[63] evaluated evidence for the efficacy of *Aloe vera* on managing prediabetes and early non-diabetes mellitus. The authors performed a systematic search of PubMed, EMBASE, and Cochrane Central Register of Controlled Trial until 28 Jan 2016. A total of five randomized controlled trial (RCTs) involving 415 participants were included. The evidence from RCTs showed that *Aloe vera* might effectively reduce the levels of FBG, HbA1c, triglyceride, TC and LDL-C, and increase the levels of HDL-C on prediabetes and early non-diabetic patients.

Roshanravan *et al.*^[64] evaluated the effects of butyrate and high performance inulin supplementation simultaneously or singly on glycemic status, lipid profile, and glucagon-like peptide 1 level in 60 adults with type 2 diabetes mellitus. In their study, group A received butyrate capsules, group B received inulin supplement powder, group C was exposed to the concomitant use of inulin and butyrate, and group D consumed placebo for 45 consecutive days. Markers of glycemia, lipid profile, and glucagon-like peptide 1 were measured pre- and post-intervention. Dietary supplementation in groups A, B, and C significantly reduced diastolic blood pressure in comparison with the placebo group. Also, intra-group statistical analysis showed that only treatment (Group C) significantly reduced fasting blood sugar and waist to hip ratio. Waist circumference in groups B and C reduced significantly after the intervention. The post hoc Tukey tests showed significant increase in glucagon-like peptide 1 concentration in groups A and C in comparison with group D. The results suggest that inulin supplementation may be useful to diabetic patients and these effects could be increased with butyrate supplement. Further, the authors^[65] investigated the role of butyrate and inulin supplements on inflammatory and oxidative stress parameters in type 2 diabetic patients. Sixty overweight and obese diabetic patients were recruited and randomly allocated into four groups. The groups received, respectively, 600 mg/d butyrate (group A), 10 g/d inulin powder (group B), both inulin and butyrate (group C), or placebo (group D) for 45 consecutive days. There was a significant increase in *Akkermansia muciniphila* percent change in inulin and butyrate supplemented groups. Furthermore, significant decrease was seen in TNF- α mRNA expression in group A, group B, and group C. Also hs-CRP, MDA and diastolic blood pressure levels decreased significantly in these groups. Intervention had significant effects on inflammatory and oxidative stress parameters and led to improvement of hypertension. The authors^[66] evaluated the effect of butyrate and high-performance (HP) inulin supplementation on the promotion of gut bacterium *A. muciniphila* growth and alterations in microRNA (miR-375)

and Kruppel-like factor (KLF) 5 expression in patients with T2D. The results showed that *A.muciniphila* percent change increased significantly after supplementation with HP inulin and butyrate. Also, supplementation with HP inulin significantly decreased the KLF5 fold change after intervention. In particular, in comparison to the placebo group, an increased expression of miR-375 was seen after butyrate and butyrate+inulin supplementation. The authors^[66] concluded that inulin and butyrate may potentially promote gut health and can be considered as novel therapeutic approach for the prevention and control of diabetes.

SUMMARY

We previously proposed the brain-liver-intestine circuit network for the coordination of insulin sensitivity in endogenous glucose production^[67]. The beneficial effects of polyphenolic ingredients with the metabolites of aloe polysaccharide, such as manno-oligosaccharide in *Aloe vera* and SCFA to gut-brain (hypothalamus)-axis of hepatic glucose production and insulin sensitivity in long term ingestion of aloe vera gel were highlighted^[68]. Moreover, we discussed the possible participation of butyrate and its necessity to determine whether an altered gut microbiota precedes development of insulin resistance and diabetes, and to identify the underlying molecular mechanisms^[69]. Ingestion of prebiotics or probiotics has been used to treat a range of conditions including constipation, allergic reactions and infections in infancy, in patients with irritable bowel syndrome. An innovative concept of symbiotic: a combination of aloe vera gel and microbiota is a perspective on modulation of insulin sensitivity and improvement of gastrointestinal health by butyrate fermentation. Daily intake of the butyric acid fermentation extract from *Aloe vera* inner gel with endophytic bacteria may provide the possible potential preventive and therapeutic approaches for human health^[23].

Better understanding of the mechanism of action of butyrate in intestinal physiology and lipid metabolism will facilitate the application of butyrate and HDAC inhibitors in gut health improvement and control, and the prevention of metabolic diseases such as obesity and insulin resistance as the effects of gut microbiome on health are multifarious. It is, therefore, expected that prebiotics or probiotics will be combined with other nutritional compounds to achieve a more robust health effect. Daily ingestion of aloe vera gel that increases butyrate levels may serve as effective means to mitigate colon cancer, T2D diabetes, and obesity.

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