Cellular and Physiological Effects of Short-Chain Fatty Acids

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Abstract: Short chain fatty acids (SCFA) have multiple *in vivo* and *in vitro* effects including cell cycle arrest and induction of protein synthesis, differentiation, and apoptosis. Butyrate or derivatives have promise for treating inflammatory bowel disease and colon cancer. Genomic and proteomic analysis have generated new knowledge on function and applications for SCFA.

Keywords: short chain fatty acids, butyrate, inflammation, cancer, colon and fiber.

OVERVIEW

The old adage "you are what you eat" may be truer than anyone ever supposed. The ongoing controversy surrounding the contribution of genotype versus phenotype to an individual's health and/or behavior is reflected by the phrase "nature vs. nurture". At one time in the not too distant past it was suggested that a person's genetic makeup could explain virtually every characteristic about an individual, especially their propensity for disease. Recently it has been recognized that the regulation of gene expression, especially by environmental factors, is as important as which genes an individual possesses. This view has come about partly because of a large body of research indicating that the nutritional state has a profound effect on general health. Thus, an organism's phenotype may be just as important as its genotype. Although an organism's genotype does not change significantly over its lifespan, with the exception of genetic mutations or rearrangements, the phenotype is influenced by many factors including nutritional state, stress, chemical agents, and the aging process. Perhaps most important, due to the fact that we constantly need to eat, are the phenotypic changes induced by an individual's choice of food. Obviously, there are a myriad of foods consumed that have the potential to affect phenotype due to the thousands of molecules that are present in them.

This raises the possibility of utilizing nutrition to improve health and counteract disease, the concept of "nutriceuticals". This concept is attractive for several reasons, which include ready availability, low cost, and the perceived benefit of ingesting a "natural" substance. The use of foods or supplements to treat pathological conditions is an old idea, one that dates back thousands of years. Recently, the molecular basis and the specific interactions responsible for the effects of these substances is becoming known, allowing us to focus on which foods to eat, or more conveniently, to use concentrated forms of these agents. The ability of various fatty acids to influence health in both a positive and negative fashion has been recognized for some time. A subgroup of dietary lipids, the short chain fatty acids (SCFA), is not much known and studied as compared to medium and long chain fatty acids. Several reasons may account for this fact. One is that until relatively recently it was not widely recognized that significant concentrations of these molecules were produced in the body. Another reason is that they occur naturally in foods, but only in low concentrations. Also, their effects on gene expression have been recognized for some time, but until recently their mechanism of action was unknown. This chapter will attempt to address current knowledge of the physiologic, cellular, and molecular effects of SCFA and their possible use for the treatment of various diseases. While not intended to be comprehensive, this review will highlight selected areas of research that have potential importance for understanding the physiological effects of SCFA. The primary focus will be on butyrate, which is the most extensively studied SCFA in terms of its range of biological effects.

SHORT CHAIN FATTY ACID ORIGINS

Fatty acids are components of saturated and unsaturated complex lipids, many of which occur naturally and are synthesized in the body. The most abundant fatty acids range in length from 12 to 22 carbons, with those of 16 to 18 carbons predominating. The most common SCFA include propionic, butyric, and valeric acids, with 3, 4, and 5 carbons, respectively, as shown in Fig. (**1**). They occur naturally in certain foods including fruits and vegetables, but the highest amounts are detected in products containing milk fat, such as butter, which contains 3 to 4% butyrate complexes as glycerides or glycerol esters [1]. SCFA also are generated transiently during the synthesis and breakdown of fatty acids, but concentrations reached are not significant physiologically except in special cases. For example, in fasting animals or in people with diabetes mellitus fatty acids are incompletely oxidized, leading to a significant accumulation of D-β-hydroxybutyric acid and acetoacetic acid (ketone bodies) in blood and urine [Fig. (**1**)]. Thus, under normal conditions, significant quantities of SCFA are neither ingested nor generated by metabolic reactions.

There is a natural primary source for the generation of large amounts of SCFA in the human body, the bacterial anaerobic fermentation of carbohydrates in the colon, where SCFA may reach millimolar concentrations [2,3]. This process is similar to the fermentation that takes place in the rumen. The major source of this carbohydrate is fiber, which

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1389-5575/04 $45.00+.00

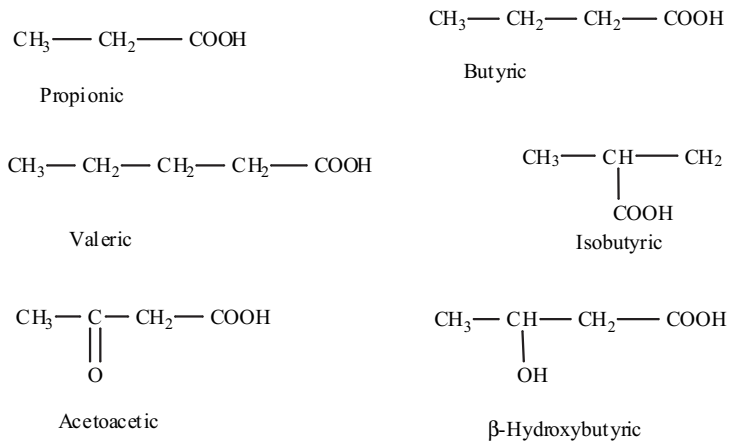

Fig. (1). Examples of common short chain fatty acids

Acetoacetic acid and β-hydroxybutyric acid often are referred to as ketone bodies.

consists of plant cell wall polysaccharides such as cellulose, pectins, and hemicelluloses. These polysaccharides are composed of hexose, pentose, and uronic acid monomers. The main metabolite produced is pyruvate, which is converted to acetate, propionate, butyrate, CO_2, hydrogen, methane, and water. SCFA are the primary anions found in feces. Starch also may be a substrate for fermentation, if present in the colon in sufficient quantities. However, such starch must survive absorption and digestion in the small intestine, and is referred to as resistant starch.

Early work by Cummings [2] showed that acetic, propionic, and butyric acid could be detected in the large intestine of all mammalian species, including herbivores, omnivores, and carnivores. Concentrations of these SCFA are controlled by the balance between production and absorption rates, and also by the transit time through the gut. Levels in the gut are affected by diet, although the extent to which this occurs varies between animals. A high carbohydrate diet causes a drop in total SCFA concentrations in feces (85 to 46 mmol/L) and feeding methylcellulose caused a further drop to 23 mM. Roediger [4] demonstrated that the majority of SCFA production, especially butyrate, is metabolized by epithelial cells in the colonic mucosa. In fact, butyrate is the primary energy source for the colon. It is interesting to note that isobutyrate also functions as an energy source for colonocytes under conditions of low butyrate availability such as starvation [5]. Isobutyrate and valerate also may be detected in feces at low concentrations (1-2 mM), however, these SCFA are derived from metabolism of branched chain amino acids, and are not generated by carbohydrate fermentation.

Because evidence for fermentation was indirect, Cummings *et al.* [3] performed an unusual and interesting study that used sudden death victims within 4 hrs of death to provide direct measurements of human fermentation products. They measured SCFA in all regions of the large intestine and in portal, hepatic, and peripheral venous blood. They found that total SCFA concentrations in mmol/kg were low in terminal ileum (13±6) and high in all regions of the colon; 131±9 in caecum to 80±11 in descending colon. Total SCFA (micromolar) in blood at the portal vein was 375±70, in the hepatic vein 148±42, and in the peripheral circulation 79±22. Acetate was the primary anion detected in all samples, and molar ratios of the three principal SCFA changed from the colonic contents to portal blood to hepatic vein, which indicates greater uptake of butyrate by colonic epithelium and propionate by the liver. These results have been confirmed by other studies in both pigs and humans (Reviewed in [6]).

Although significant concentrations of SCFA such as butyrate are produced in the gut, only very low concentrations may be detected in the peripheral circulation. Moreover, even alterations in diet that significantly increase production of SCFA in the gut have an insignificant effect on circulating concentrations of butyrate. Thus, it seems unlikely that naturally occurring butyrate could have a major effect on gene expression outside the gut, with the notable exception of periodontal disease [7]. Administration of exogenous butyrate may be a possibility, however, and this will be discussed in more detail in the section on cancer. The following sections will consider effects of SCFA, primarily butyrate, on intestinal dysfunction, inflammation, thromboresistance, and cancer.

CELLULAR AND PHYSIOLOGICAL EFFECTS OF BUTYRATE

A large body of work exists which demonstrates that SCFA can induce a wide variety of reversible effects on cultured cells. Early work showed that butyrate could inhibit cell growth, induce protein and enzyme synthesis, and arrest the cell cycle [8]. Butyrate also induces differentiation and apoptosis in a variety of *in vitro* models at concentrations greater than 50 to 100 μM. Riggs *et al.* [9] showed that butyrate could act as a histone deacetylase inhibitor, and this effect is thought to be the mechanism for many of the effects of butyrate. Butyrate has the most potent effects of any of the SCFA, and this appears to be related to its straight-chain four-carbon structure. A straight-chain four-carbon monocarboxylic acid is the most effective configuration for inducing hemoglobin [10], choriogonadotropin [11], and fibronectin synthesis [12], indicating a strong relationship between structure and function. It should be noted that specific effects of SCFA are concentration dependent.

Propionate may have effects on cell proliferation similar to butyrate if a sufficiently high concentration is used.

INTESTINAL DYSFUNCTION

Not surprisingly, SCFA have many effects upon the intestine. One example is the use of fiber to treat intestinal dysfunction. Patients with severe intestinal dysfunction usually receive a nutritional regimen of bowel rest consisting of intravenous total parental nutrition (TPN). This regimen results in atrophy and hypofunction throughout the gastrointestinal tract. These effects may be successfully reversed by enteral feeding. Most liquid formula elemental diets have no fiber, leading to colonic atrophy. The addition of fiber to the formula prevents the occurrence of atrophy. Pectin is a soluble dietary fiber that has been studied for its ability to counteract atrophy. Pectin is a non-cellulose fiber polysaccharide composed primarily of galacturoic acid, and is fermented in the gut to produce SCFA. It has many effects upon the gut which include increased lipase activity, intestinal transit time and SCFA production, and helps to maintain the morphology and structure of intestinal villi, and to delay gastric emptying time (Reviewed in [13]). Pectin has also been shown to improve healing of experimental colonic anastomoses in the rat when added to an elemental diet. Criteria used to assess healing include bursting pressure and collagen synthesis [13]. Pectin may stimulate mucosal growth, and this effect has been shown to enhance intestinal adaptation following intestinal resection. Presumably these effects are at least partly attributable to increased concentrations of SCFA such as butyrate.

INFLAMMATION

A primary event in any inflammatory process is the activation of the endothelium. Endothelial activation is characterized by increases in the expression of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin [14], and human leukocyte antigens such as HLA-DR. Increased ICAM-1 expression, alone or in combination with other markers such as HLA-DR, is associated with coronary artery disease [15,16], diabetic retinopathy [17], and ischemic stroke [18]. It is considered a marker of vascular inflammation [19], and may be used to assess endothelial cell activational status in a variety of vascular diseases. Adhesion molecules are involved in the regulation of leukocyte trafficking across endothelium [20)]. Early ICAM-1 expression on arteriolar endothelium has been shown to be related to the development of coronary artery disease, as well as to the severity of arteriopathy in transplanted human hearts [16]. Mediators of endothelial activation include molecules such as cytokines, endotoxins, thrombin, antibodies, and lysophosphatidylcholine [14,21-24].

Butyrate has been shown to induce ICAM-1 expression on a few types of cells. Perrin *et al.* [25] studied the combination of interleukin 2 and sodium butyrate as immunotherapy for colon cancer peritoneal carcinomatosis. They found that butyrate increased expression of ICAM-1 on rat colon cancer PROb cells *in vitro*, as well as in established PROb peritoneal carcinomatoses following intraperitoneal injections. Cases of total regression of tumors were noted, and attributed, at least in part, to increased butyrate-mediated immunogenicity. Butyrate also has been shown to increase ICAM-1 expression on SW 1116, HT-29, and HT-29 Glc human intestinal epithelial cell lines, but not on Caco2 or HT-29 MTX lines [26]. Maeda *et al.* recently demonstrated that ICAM-1 and HLA-DR expressions were increased on clinical samples of acute myeloid leukemia cells treated with sodium butyrate [27]. However, the ability of butyrate to induce ICAM-1 expression is not universal. For example, human colonic adenocarcinoma cells (HT-29m3) do not upregulate ICAM-1 when cultured with butyrate concentrations up to 8.0 mM [28], suggesting that butyrate-mediated ICAM-1 expression is cell specific. It is not clear if an increased circulating level of butyrate, whether due to endogenous or exogenous sources, is capable of inducing ICAM-1 expression.

Diabetes is associated with defects in the metabolism of fatty acids [29] and this results in increased production of ketone bodies such as β-hydroxybutyrate. Vascular disease is also associated with diabetes, and is characterized by endothelial upregulation of ICAM-1 [17]. However, there is no evidence available to suggest that β-hydroxybutyrate affects adhesion molecule gene expression in diabetes. A recent study did show that an infusion of β-hydroxybutyrate was able to inhibit myocardial fatty acid oxidation, and that this effect was not related to changes in malonyl-CoA levels [30]. Possible diabetic-associated changes in SCFA may be worthy of further study, as it is not clear what other SCFA may be produced by diabetics, and in what concentrations they are present in the circulation.

Although the concentration of butyrate in the general circulation normally is in the low micromolar range [3], there are instances where butyrate may have a significant physiological role. Butyrate and propionate have been implicated in the pathogenesis of periodontal disease related to their ability to inhibit endothelial cell proliferation. Their concentrations in dental plaque fluid can reach 20 to 27 mM [7]. Tse *et al.* [7] have speculated that butyrate may be involved in regulating endothelial cell adhesion molecule expression. Intrahepatic delivery of butyrate at millimolar concentrations has been shown to increase Kupffer cell production of prostaglandin E2 and augments the immunosuppressive effects of portal venous transfusions [31]. Effects of butyrate on localized vascular expression of hepatic ICAM-1 have not been demonstrated, but are a possibility.

It is interesting to speculate on the effects that small increases in circulating concentrations of SCFA may have on the vasculature. It is possible that slight but sustained increases in SCFA concentration could lead to a low level of endothelial activation, and such cellular activation has been suggested as a possible risk factor for cardiovascular disease [32].

An obvious location where changes in SCFA concentrations could affect inflammation is in the colon, where high concentrations are known to exist and where the concentrations may be easily modulated. There are several lines of evidence to suggest that SCFA such as butyrate may have anti-inflammatory actions in the colon. The source of most complement components has been thought to be the liver hepatocytes, but recent evidence suggests that C3 and factor B transcripts are expressed in normal human colonic

epithelial cells [33]. C3 and factor B form the C3 convertase complex of the alternative pathway. Induction of complement C3 and factor B enhances local immunological defense activity, but also enhances alternative pathway-mediated cytotoxicity to epithelial cells. Butyrate strongly inhibits TNF-alpha-induced factor B biosynthesis that may block alternative complement activation, thus reducing cytotoxic activity. C3 and factor B genes contain NF-kB and C/EBP binding sites in their promoters, which drive their expression. Sodium butyrate inhibits TNF-alpha stimulation of induction of NF-kB p50/p65 heterodimer DNA binding complex; whereas it increases TNF-alpha induced C/ERBβ DNA-binding activity.

Other studies have shown that butyrate can inhibit basal and LPS-mediated cytokine secretion from mononuclear cells isolated from patients with Crohn's disease [34]. This effect was associated with the inhibition of NF-kB translocation into the nucleus. Butyrate also can inhibit TNF-alpha-mediated NF-kB activation in HT-29 cells by blocking IkB-alpha degradation [35]. However, in HeLa229 intestinal epithelial cells IkB-alpha phosphorylation and degradation was not inhibited by butyrate although it still inhibited IL-1-mediated NF-kB activation. These results provide further evidence for the hypothesis that the effects of butyrate are cell specific, and parallel the results previously discussed for ICAM-1 activation.

Butyrate may have application in the treatment of inflammatory bowel disease [33]. The use of butyrate enemas has been shown to significantly decrease TNBS-induced experimental ulcerative colitis, and also to reduce inflammation in ulcerative colitis patients, although to a lesser extent. A typical enema consists of 80 mM acetate, 30 mM propionate, and 40 mM butyrate. The use of enemas with a higher butyrate content, or perhaps composed entirely of butyrate, may prove to be even more effective.

THROMBORESISTANCE

A small amount of data is available on the effects of butyrate on the expression of molecules related to thromboresistance of the vasculature. Kooistra *et al.* [36] showed that butyrate stimulated synthesis of tissue-type plasminogen activator (t-PA) in human umbilical vein endothelial cells. The increase in protein in the medium was time and concentration dependent, with a maximal effect at 5 mM. Relative changes in t-PA production ranged from 6- to 25-fold in various endothelial cell cultures. Cells cultured 24 hours in the presence of 5 mM butyrate also had a 30-fold increase in t-PA mRNA as measured by Northern blotting. In contrast, butyrate had essentially no effect on either protein or mRNA expression of plasminogen activator inhibitor type-1 (PAI-1). The specificity of butyrate on t-PA expression was tested by incubating cells with a wide variety of SCFA and other variously modified short carbon chain compounds. The results are summarized in Fig. (**2**). Butyrate had by far the greatest effect on t-PA synthesis, with valerate and propionate showing much less activity, at least at the same concentration. Acetate, as well as terminal modifications such as alcohol or amino groups, or substitutions such as a methyl group for the terminal carboxy group, almost completely eliminated activity. Butyrate, as previously noted, seems to possess a unique structural specificity, and this has been noted for the induction of synthesis for other proteins as well. In another study, butyrate-mediated stimulation of t-PA synthesis was

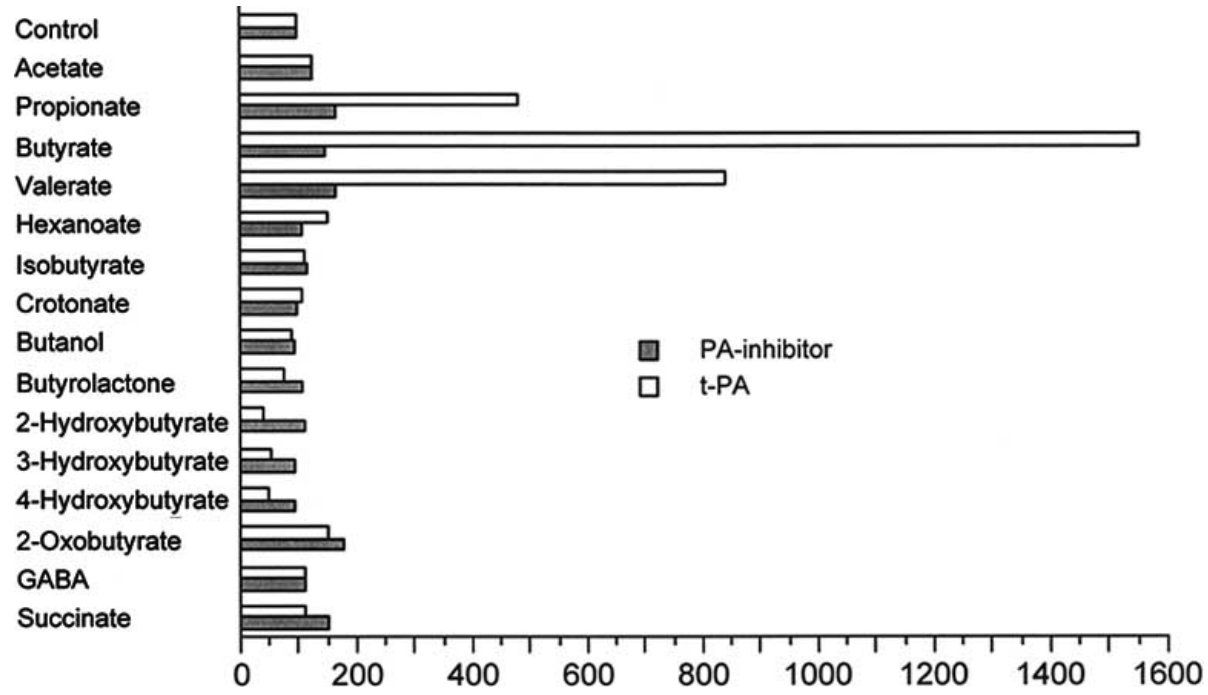

Fig. (2). Structural specificity of butyrate effect

Endothelial cells were incubated with various butyrate analogues (5 mM, 24 h), and the conditioned medium were analyzed for t-PA antigen and PAI-1 activity. Abbreviation: GABA, 4-aminobutyrate.

Adapted with permission, from Kooistra *et al*., 1987, Biochemical Journal, 247, 605-612. © the Biochemical Society.

related to histone acetylation [37]. Butyrate increased t-PA production on a transciptional level and the effect of butyrate was mimicked by trichostatin A, a specific inhibitor of histone deacetylation. The effects of butyrate and trichostatin A were not additive, suggesting a common mechanism, and t-PA induction was preceded by acetylation of histone H4. However, histone acetylation could not explain 100% of the effect, and thus other factors are responsible for the actions of butyrate.

In addition to fibrinolysis, butyrate may affect expression of molecules involved in natural vascular anticoagulant pathways. Justus *et al.* [38] showed that incubation of HUVEC with 5 mM butyrate resulted in an increased ability of the cells to bind antithrombin. The effect was time and concentration dependent, and as was the case for t-PA, butyrate had the most activity compared to isobutyric, propionic, or valeric acid. Butyrate increased sulfation of heparan/heparin sulfate which in turn increased its affinity for antithrombin. Although expression of syndecan-1 or glypican was not altered by butyrate, it is possible that other HSPGs may be affected. Both of these effects have implications for countering the vasculopathies that occur spontaneously or following procedures such as cardiac transplantation. The problem with administering SCFA as a potential therapy is achieving sufficient concentrations in the peripheral circulation. This is discussed in more detail in the following section on cancer.

CANCER

In Vitro Studies

Butyrate, and to a lesser extent other SCFA, have been shown to possess several properties that make them possible anti-cancer agents (Reviewed in [39,40]. These include inhibition of proliferation, stimulation of apoptosis, and differentiation activity. These effects may be explained at least in part by the ability of butyrate to inhibit the activities of many histone deacetylases, resulting in hyperacetylation of histones. This action disrupts chromatin structure, allowing greater access of polymerases to selected regions of DNA [41] and thus increases gene expression. Inhibition of histone deacetylases is associated with regulation of molecules key to the cell cycle such as cyclin D, p21 and p27, although the exact mechanism responsible has yet to be completely elucidated. A partial explanation may be provided by recent results from Merchant *et al.* [42], which show that the zinc-finger binding protein-89 is a butyrate-regulated coactivator of p53 and induces cyclin-dependent kinase inhibitor p21 (Waf1). Other histone deacetylase inhibitors include trichostatin A (fungistatic antibiotic from Streptomyces platensis), depsipeptide (FR901228, FK228), valproic acid, and suberoylanilide hydroxamic acid. Butyrate is particularly attractive due to low cost, ready availability, and established safety. Synergistic effects of butyrate and DNA methyltransferase inhibitors such as 5-aza-cytidineand 5-aza-2'-deoxycytidine against cancer cells have been found in human lung, breast, thoracic, leukemia and colon cancer cell lines [39].

Although most studies on the anticancer activity of butyrate have focused on its ability to inhibit histone deacetylases, it is possible that butyrate could exert its effects through other mechanisms. For example, message for both the *sis* proto-oncogene [43] and tissue-type plasminogen activator [37] were increased by butyrate, and this effect was due to a significantly increased stability of the mRNA. Direct effects of butyrate on gene transcription also may occur. Butyrate has been shown to modulate the expression of suppresser and proto-oncogenes in several cell types [43-47] including the *fos* and *jun* proto-oncogenes, the gene products of which combine to form the activator protein-1 (AP-1) transcription factor. In fact, butyrate has been shown to induce c-fos transcription in the Caco-2 colon adenocarcinoma cell line by acting on the ATF-CRE binding site and increasing CRE binding [45]. At least one instance is known where butyrate apparently acts to derepress gene transcription [48]. Evidence also exists for a butyrate response element for 5'-flanking sequences, which have been shown to mediate the stimulation of embryonic globin gene expression by butyrate [49], and a sequence element that confers a butyrate-inducible response has been identified in the 5'-flanking region of the mouse calbindin-D28k gene [50]. More work will need to be done to elucidate the contributions of these mechanisms to butyrate-mediated modulation of gene expression.

In Vivo Studies

Due to the encouraging results from laboratories studying the antiproliferative actions of butyrate, it is not surprising that attempts have been made to use SCFA in the clinic to treat various cancers. Although butyrate has shown promise as an antineoplastic agent, it has pharmacokinetic characteristics that make its use problematical (Reviewed in [1]). In order to be effective as a differentiating agent, continuous, high levels of butyrate are required to cause effects on cancer cells. For effects *in vitro*, concentrations of at least 0.05 mM are needed for red blood cell changes such as sickle cells. Minimum concentrations of 0.3 mM are needed for leukemia cells and colon cancer cells, and levels of 1-5 mM have been shown to be more effective. The assumption is that the same concentrations are needed *in vivo*, but these are much more difficult to attain. Plasma clearance of butyrate is very rapid, with a half-life in the order of 6 min when given intravenously. Several trials have been performed, with varying success, that used 500 mg/kg per day of the sodium or arginine salt of butyrate. Arginine salts have been used in an attempt to circumvent negative effects from the increased sodium load when using large amounts of sodium salts. Blood levels did not rise above 0.05 mM in any of the studies, a concentration most likely insufficient to stimulate differentiation.

To circumvent problems with rapid metabolism of butyrate, stable derivatives or prodrugs of butyrate have been studied both *in vivo* and *in vitro* for possible use as antineoplastic agents. Tributyrin is a prodrug consisting of a triglyceride backbone with three butyrate moieties esterified to glycerol. Butyrate's differentiating ability is probably due to histone deacetylase inhibition resulting in induction of cyclin-dependent kinase inhibitor p27 and consequent downstream effects on Rb phosphorylation. Tributyrin has been shown to have similar activities as butyrate, including antiangiogenic effects [51], apoptosis effects [52], and *in vitro* synergism with retinoids [51,53] and interleukins [54].

Other BTR derivatives are being developed such as pivaoyloxy methyl butyrate [55]. When tributyrin was administered orally as opposed to intravenously, the half-life was increased to 40 min., and circulating butyrate concentrations reached a maximum of 0.34 mM, high enough for efficacy. Daily oral doses of tributyrin have been reported to produce circulating levels >100 μM for 0.5 to 4 hour periods. The reason for the increased half-life with oral compared to intravenous administration of tributyrate is not clear, but may be related to the hydrolysis process, the extended release time through the gut, or inherent properties of tributyrin. Other studies have utilized phenylacetate, but it has an objectionable odor. Phenylbutyrate, another butyrate prodrug, has a much less objectionable odor, and similar effects to butyrate.

For butyrate to be effective as a differentiating agent circulating concentrations would need to be maintained at a consistently high level. This is difficult to achieve because of its short half life, but a possible solution would be to give multiple daily doses. Edelman *et al.* [56] evaluated a three-times daily dosing schedule. Patients (n=20) with advanced solid tumors were treated with 150 to 200 mg/kg three times daily. Blood was sampled at 1, 1.5, 2, 2.5, 3, 3.5, and 4 hr. after administration. No dose-limiting toxicity was seen. A moderately high butyrate average concentration of 52 μM was attained, but there was high variability between patients. No statistically significant outcome occurred, but a few patients had increased survival.

The most logical application for the antineoplastic effects of butyrate would seem to be in the colon, where high concentrations can be readily achieved. Indeed, such studies have been done. Many studies have suggested that diets high in fat and/or protein may contribute to an increased incidence of colon cancer. In contrast, diets with high percentages of fiber and/or complex carbohydrates may decrease the occurrence of such cancers. Increased concentrations in the colon of SCFA such as butyrate have been shown to correlate with dietary fiber-associated protection from colon cancer. Effects of butyrate on colon cancer cells reflect the results of previous studies on other cell types, and include inhibition of cell proliferation, induction of differentiation, and promotion of apoptosis. Growth inhibition of colon cancer cells appears to be linked to histone hyperacetylation and subsequent induction of cell cycle inhibitor p21 and cyclin D3 expression, which arrests them in the G1 phase [57]. Another mechanism by which butyrate may inhibit cancer cell growth is its ability to retard cell adherence to the basement membrane protein laminin [58]. Colon cancer cells bind to laminin via fibronectin or type IV collagen, and butyrate may disrupt this process by direct or indirect means.

Other effects of butyrate also have been reported. Emenaker *et al.* [59] showed that butyrate inhibited urokinase plasminogen activator (uPA) expression and stimulated tissue inhibitor matrix metalloproteinase (TIMP)-1 and -2 protein expression. Because uPA and matrixmetalloproteinase (MMP) activity are linked to metastasis of tumor cells, these results suggest butyrate may be effective in inhibiting the invasiveness of colon cancer cells. Butyrate has been shown to reduce the expression of the caspase inhibitor known as Flice-like protein [60], which may explain part of its known effects on stimulating apoptosis. Butyrate also acted synergistically with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), suggesting that the combination may increase their individual effectiveness in promoting colon cancer cell death.

Another mechanism that may allow butyrate to protect against colon cancer cells is that of increasing immunosurveillance (see [61] for review). Butyrate enhanced the immunogenicity of PROb cells and increased their sensitivity to IL-2-activated natural killer cells by upregulating expression of ICAM-1 [25]. Butyrate also has been shown to decrease expression of decay-accelerating factor (DAF) in several colon cancer cell lines. DAF on the surface of cancer cells behaves as a barrier to removal of the cells by complement activation [33].

Recent advances in high-throughput analysis of gene and protein expression have been utilized to study the effects of butyrate on colon cancer cells (Reviewed in [62]). Many studies using microarrays to investigate effects of butyrate and other compounds on cancer cells have been performed, but the first is credited to Mariadason *et al.* [63]. This group used microarray analysis to study the effect of 5 mM butyrate on the SW620 colon carcinoma cell line compared to the histone deacetylase inhibitor trichostatin A (TSA), sulindac, a non-steroidal anti-inflammatory drug, and curcumin, a chemoprotective agent known to inhibit AP-1. Six time points up to 48 hours were analyzed following the treatment of the cells. Among many interesting results was the observation that although phenotypic responses such as induction of cell cycle arrest and apoptosis were similar for butyrate, TSA, and sulindac, gene expression patterns were in some cases quite different. This result suggests that the compounds act though different pathways, raising the possibility for synergistic effects, which might be useful in clinical treatment protocols. At least one proteomic study of the action of butyrate on colonocytes has been performed [64]. Alterations in expression for proteins involved in the cell cycle, apoptosis, and transcriptional regulation were detected, which correlate with previous studies, but effects on components of the ubiquitin-proteasome pathway were also noted which may provide a novel mechanism for some of the previously described effects of butyrate. Although all the studies done to date utilizing genomic and proteomic analyses of butyrate–mediated effects on colon cancer cells have differences in their experimental protocols, common results show suppression of cell growth and induction of apoptosis, most likely due to histone hyperacetylation. These techniques will be of great value in future studies designed to test hypotheses of the mechanisms responsible for the many actions of SCFA such as butyrate on cells and tissues.

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